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Journal of DENTAL RESEARCH

Founded by William J. Gies in 1919

OFFICIAL PUBLICATION OF THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Editor

HAMILTON B. G. ROBINSON

AUGUST, 1951

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Organized: New York City, Dec. 10, 1920. Of those at the meeting of organization, the following remain active members: Adolph Berger, Theodor Blum, G. W. Clapp, H. S. Dunning, W. J. Gies, A. H. Merritt, B. B. Palmer, L. M. Waugh.

Objects (Const., Art. II): . . . "(a) To promote broadly the advancement of active research in all branches of dental science and in related phases of the sciences that contribute directly to the development of dentistry, and which add to the knowledge of the mouth and teeth and of their relations to the body as a whole; . . . (b) to encourage and facilitate coöperative effort and achievement by, and mutual helpfulness among, investigators in all nations in every division of stomatology; (c) to the end, particularly, that dentistry may render cumulatively more perfect service to humanity." (The Association has been using "dentistry" and "stomatology" as synonyms.)

Membership: total (Nov. 10, 1946), 627. *Eligibility (Const., Art. 14, Sec. 1).*—"Any person who has conducted, and published an account of, a meritorious original investigation in dental science, or in any of the sciences contributory to stomatology, shall be eligible to membership in this Association, providing such person conforms to the recognized standards of professional ethics." *Election.*—New members may be elected *only at general meetings of the Association.* Membership in a section is secondary to membership in the Association as a whole. Removal of members from one geographic center to another does not affect their membership in the Association.

Endowment fund of the International Association for Dental Research (as of June 1, 1949): $1,506.06. There have been no expenditures from this fund.

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The JOURNAL OF DENTAL RESEARCH was dedicated by its founder to the dissemination of knowledge pertaining to the teeth and mouth, and to their relations to the body as a whole. It publishes original investigations in these and related fields. Articles are accepted only on condition they are contributed solely to this JOURNAL. Articles appearing in other domestic or foreign periodicals will not be reprinted in the JOURNAL except in extraordinary cases. The JOURNAL will reproduce a limited number of half-tones or zinc etchings without cost to the author. References to the literature must conform to the following style:

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THE EDITOR'S VIEWPOINT

The Proceedings of the General Meeting of the International Association for Dental Research published in this issue constitute a report on dental research in action. Ninety-nine papers were presented at the regular sessions, fourteen at the Materials Group Meetings and an additional fifty-five are printed as "read by title." These cover a variety of subjects ranging from the basic sciences to clinical applications. The abstracts are advanced reports on current or recent investigations and differ from the usual abstract which is published as a summary of previously published material. These abstracts are worthy of careful reading. Many of the investigations published in this form in the JOURNAL over the past years have never been published in any other manner. For example, this year's abstracts supply answers to many questions, including the following:

Has any community reported adversely on the results of artificial fluoridation of water supplies? (Abstr. 17)

Have in vitro lesions of caries been produced? (Abstr. 66)

Is cortisone being studied in respect to oral lesions? (Absts. 6, 78, 79)

What are the latest reports on ammoniated dentifrices? (Absts. 31, 73, 95, 99, 117, 148)

Are self-curing resins effective restorative materials? (Absts. 103, 104, 105, 106, 168)

Are the side-effects of local anesthetics psychic or toxic? (Absts. 8 and 134)

Is aureomycin effective in treatment of acute herpetic gingivo-stomatitis? (Abstr. 1)

Which type of dental instrument leaves the smoothest cavity wall? (Abstr. 45).

This year you have the opportunity to read these reports less than six months after their presentation at French Lick Springs. Perhaps we can publish them as early as three months after the meeting.

Although vitamin deficiences of oral acidogens may account for reduced caries activity in vitamin deficient human subjects, the vitamin requirements of oral bacteria have not received much attention. By adjusting the vitamin content of a basal media the vitamin requirements of bacteria can be accurately studied. Koser, Fisher, and Kauffman (p. 532) have investigated the need of certain strains of lactobacilli for some of the B complex factors, including riboflavin, nicotinic acid, biotin, calcium pantothenate, folic acid, pyridoxamine hydrochloride, pyridoxal hydrochloride and thiamine.

Pearlman and Hill (p. 542) have intensively studied the influence of ammonia and urea on a strain of lactobacillus. They found the inhibitory activity of ammonium markedly dependent on many factors that can be varied in vitro. The inhibitory action of urea, they suggest, is more likely to be a result of its denaturing action on protein than an its alkalinity or ammonia production. They do not draw conclusions as to the possible effectiveness of ammonia and urea in inhibiting caries. This basic laboratory study utilizes carefully controlled techniques and as a result can be used to explain some variations apparent in less exacting studies.

Cobe and Lederknight (p. 558) propose the use of quaternary ammonium compounds as anticaries agents in dentifrices. Preliminary tests indicate that they inhibit the oral flora but not lactobacilli in particular. The reduction of the total oral flora by 50 to 60 per cent three hours after use of the compounds may or may not be significant. It is more likely that standard caries susceptibility tests given at regular intervals after beginning daily use of these compounds, would supply useful evidence. The final test is always whether the compounds actually inhibit caries.

Losee, Leopold, and Hess (p. 565) recovered approximately 15.5 per cent protein from dentin. This is lower than proposed values of 21 to 22 per cent but, as the authors point out, their figures give the recoverable maximum, while the higher values are for the predictable maximum. These studies of the organic content of dentin are highly important in continuing development of our knowledge

of teeth. Sometimes the clinical value of these investigations may not be clearly apparent. It is extremely interesting that this basic work is jointly supported by the U. S. Navy and a university.

Levy and Detwiler (p. 575) observed the development of the mandibles of salamanders after transplantations and operations, reversing the axis of the arch on one side. In the transplanted mandibles teeth developed, but new mandibular arches did not develop in the embryos from which the transplants had been removed. Their observations led them to support the conclusions of others in the field of comparative anatomy and zoology that the teeth and cartilages of the mandibular arch are derived from cells of the neural crest. The number of papers on comparative dental anatomy appearing in the dental and other scientific literature has been quite scanty in recent years. This interesting field holds much of promise to investigators of normal and pathologic processes.

Zimmerman and Zeidenstein (p. 587) observed the origin of the minor (labial, buccal, retromolar) salivary glands in the human fetus and studied their distribution. These glands have been overlooked to a large extent, although they may be of physiological importance and certainly of pathological significance in the formation of retention cysts and mixed salivary gland tumors. However, the observation of the origin of such glands hardly justifies support of the old theory that labial glands are etiological factors in enamel erosion.

H. B. G. R.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Proceedings of the Twenty-Ninth Annual Meeting
French Lick Hotel, French Lick Springs, Ind.
March 17, 18, and 19, 1951

Compiled by Hamilton B. G. Robinson, Editor, *College of Dentistry, The Ohio State University, Columbus, Ohio*

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I. Presidential Address: Remarks of Retiring President

B. G. Bibby,

Eastman Dental Dispensary, Rochester, N. Y.

Until recently, it seems to have been customary for the retiring president, as his last official duty, to make some comments on the affairs of the Association. In attempting to reestablish that usage, it is my hope that, as a result, throughout the years the members of the Association will be provided with a varied commentary on the progress, objectives, and needs of the Association.

In undertaking this self-imposed assignment, I can do no better than read a letter which I recently received from Dr. W. J. Gies, the founder and long-time secretary of our Association. In addition to providing us with happy news about his return to health following a major abdominal operation, Dr. Gies' letter not only records progress of the Association over a longer period of years than I can review, but it also restates some of the objectives which were in our founder's mind when the Association was formed. It states:

> "Since the establishment of the I. A. D. R., 30 years ago, the Association's proceedings have recurrently promoted inquiries into the validity of many aspects of conventional dental opinions. The proceedings also have encouraged the endeavors and have supported the matured judgments of the steadily growing number of ardent workers in dental research. Consequently, the Association has been a cumulatively constructive factor in the evolution of the sciences and arts of oral health service, and in the increase of the dental profession's proficiency in giving the public the most effectual dental health care.
>
> "The constructive influences of the I. A. D. R. have been strengthened by the Association's organization—from the beginning—as a federation of active geographical sections, and by the ensuing transmission of the scientific import of the general transactions to the local

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The meetings of the International Association for Dental Research were coordinated with the meetings of the American Association of Dental Schools and Omicron Kappa Upsilon.

The Editor wishes to express his appreciation to Dr. Edward H. Hatton, General Secretary, for cooperation in preparation of these Proceedings and to Dr. H. Sicher for translating the abstracts of the contributions of the Vienna Section.

groups of members, and vice versa. The influence of this intimate coordination within the Association has been extended by close correlations of the annual meetings of the I. A. D. R. with the American Association of Dental Schools, which present specially favorable yearly opportunities for both teachers and investigators to compare their understandings, responsibilities, and mutual suggestions, for the advancement of dental research, and also for the improvement of teaching in the dental schools.

"My long-standing appreciation of the accomplishments of the I. A. D. R., and my anticipations of its potential achievements, account for my enthusiastic interest in the Association's growth in size, strength, initiative, and influence. . ."

The foregoing letter indicates that the growth of the Association has been healthy and that most of its original objectives are being fulfilled in a satisfactory manner; however, growth in size gives rise to increased responsibilities and it complicates administration. In the past our secretary-treasurer has managed the affairs of the Association without assistance, but if we envision much further growth, arrangements should be devised which will take some of the responsibility from his shoulders. As a beginning step in that direction it would be useful to have officers of the Association who are better informed as to the management of the Association than I was when I took office; thereby, they might be in a position to help the secretary when they took office rather than to harass him by making mistakes or promoting policies which may only recently have been discarded. As a means of saving the secretary from having to educate the incoming generation of officers during the next year on the matters which mystified me, I initiated the practice of sending copies of all business correspondence to the president-elect and the vice president. This simple arrangement is probably only one of several devices which could be used to increase administrative efficiency.

Another method for laying a foundation for an expanded and efficient Association is to develop committees which function in a really effective way. The Association has two permanent or standing committees, the Publication Committee and the Program Committee both of which are established by the Constitution and By-laws. It also has a group of continuing or, more accurately, "repeat" committees such as those on Membership, Dental Research, and Dental Science and Art. The duties of these committees, the conditions of membership, the methods of reporting, or the reason for existence of some of them are not too certain. After taking office last year I appointed new members to all of these committees, but so that the incoming officers would come to know more about their organization, I appointed them to as many committees as possible. Out of respect for the past, and because it must be assumed that at some time all of these committees had a significant purpose, it is probable that your new president will make new appointments again this year. There is yet another group of what may be termed "moribund" committees which have been set up by action of the council in recent years. These include what was designed to be a continuing Committee on Business and Finance, the membership of which would hold office for 5 year periods. Other committees were appointed for the organization of symposia, the providing of better programs, and such purposes. The forgotten existence of these committees gives point to the statement that committees resemble illegitimate children; too often they are conceived in moments of excitement to be forgotten as soon as they are born.

It would seem that a contribution could be made to the efficiency of the Association if the activities of our committees were critically examined with the

idea of redefining their functions and procedures. Some committees, such as the Membership Committee, which have continuing responsibility might, by amendment of the By-laws, be given the dignity of becoming standing committees of the Association; the others might be given an informal burial.

The Program Committee assumed added responsibility this year in arranging the program of the annual meeting. Such responsibilities could be expanded. For instance, now that it has become the responsibility of the I. A. D. R. to select, on alternate years, a location both for its own meeting and that of the American Association of Dental Schools, that duty might be assigned to the Program Committee, thus removing a recurring item from the crowded agenda of the council.

The Publication Committee was set up for the purpose of publishing the *Journal of Dental Research* for the Association. It has functioned very satisfactorily and were it not for the hard work and devotion of the editor and his Publication Committee, the Association would not now have the excellent journal that it has. However, times have changed since the Publication Committee was created. The problems of publication, business management, advertising, and circulation are no longer in the hands of a geographically united group of Dental Fellows at the University of Rochester; instead, most of these matters are handled by the Mosby Company with whom the Association has signed a publication contract. This reduction of the Publication Committee's responsibilities in business management and circulation has not been accompanied by a reduction of their financial responsibility to the Association. Over the years the Publication Committee has become the disbursing agent for progressively greater percentages of the Association's funds. At the time the Publication Committee was formed, none of the membership dues of the Association were designated for its use. Later, in response to a request from the Publication Committee, the dues of the Association were increased to include a compulsory subscription to the *Journal of Dental Research*. Thus, the larger portion of the funds collected by the Association became assigned to the Publication Committee. This year we have had a further increase in the Association dues and the larger portion of that increase will be expended by the Publication Committee. Although there are no doubts that the expenditure will be careful and wise, it is important to note that over the years an anomalous situation has grown up under which a committee of the Association is responsible for the major financial obligations of the Association. This is not a businesslike arrangement, and it is one for which the trustees and other officers can well be criticized on the basis of administrative principle.

It is my feeling that one or two of the General Officers of the Association in addition to the Editor should automatically be members of the Publication Committee. Such a change would overcome an organizational anachronism without interfering with the authority of the editor in the running of the *Journal*. The by-products of such a reorganization would be to add greater dignity to the Publication Committee, to give more direct representation of the Association in its major financial commitment, and, in line with the need for better informed officers, it would help to educate a rotation of officers in the major business commitment of the Association. An important benefit to the *Journal* would be the existence of a larger group of officers and members in the Association who understood its publication problems, and who would be in a better position to help if the future should bring difficulties, or special opportunity.

These considerations suggest that changes in our Constitution and By-laws might be necessary. Apart from anything which has been suggested here, it seems to be time that our Constitution and By-laws were reexamined.

It has now been ten years since they were last reviewed. During that time, conditions such as those touching on the financial commitments to the Journal have changed, and, over the years, the officers and others have noted a number of ambiguities and incongruities in the By-laws. Some of these are being taken care of by amendments, but it does not seem too soon to reexamine the whole constitutional structure of the Association, to see if it cannot be made more appropriate to the conditions of today.

It is obvious that in examining the needs of the Association I have also made recommendations, however, to give these ideas a little more point, it might be worth restating them in a different form. To my successors in office I would suggest that a study be made of duties of our officers and committees for the purpose of making recommendations which would help them to work more efficiently. Such a study should include an examination of the organization and responsibilities of the Committees established by the Constitution and By-laws, and should recommend any changes in the Constitution and By-laws as should seem to be necessary to bring the desired ends.

Now that my last official statement to the Association is about to be concluded, I would like to thank the membership for the honor they have bestowed upon me by allowing me to serve as president, and to thank everyone for the support that has been given me during my tenure of office. Finally, may I convey to my successor, Dr. Fosdick, my congratulations and good wishes as he takes office. I am sure that he will find as I have, that it is a privilege to serve this very worthy organization.

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III. First Session: Morning, March 17; Abstracts 1-11

1. Acute Infectious Gingivo-Stomatitis (Herpetic-Stomatitis) of Children and its Response to the Systemic Administration of Aureomycin. *C. R. Castaldi and G. H. Rovelstad, Northwestern University Dental School, Chicago, Ill.* Numerous and various methods of treatment have been prescribed for this disease of children which has been shown to be associated with the virus, herpes simplex. Since these methods have not materially changed the course of the infection, a study was undertaken for the purpose of demonstrating the effectiveness of a known antivirus agent, aureomycin, in treating the infection. Fifty-three cases involving children from 1½ to 10 years of age have been treated during this controlled study at Children's Memorial Hospital in Chicago. There appears to be a decided response of the infection to this therapy.

2. A Preliminary Report on the Relationship of Psychosomatics to Oral Conditions. II. Relationship of Personality to Periodontal Condition. *John H. Manhold, Dental Department, Great Lakes Naval Training Center, Illinois, and Vivian W. Manhold, Phillips Academy, Andover, Mass.* A random group of 50 subjects having periodontal disease were selected by clinical examination from persons being processed through Great Lakes Naval

Training Center on return to active duty. Persons with gross malocclusion or other similar contributary defects were excluded. The individuals selected were subjected to a psychological test (Bernreuter Personality Inventory) and a complete clinical and roentgenographical survey. Statistical analysis of results showed that persons who were categorized in the neurotic tendency percentiles above the third quintile presented the more severe or Class II periodontal conditions, with but 2 exceptions. All subjects but 12 below this level exhibited cases of a Class I, or lesser, severity. The results obtained from the introversion-extroversion scoring also showed only 2 persons with a lesser periodontal condition above the upper limit with 16 exhibiting more pathologic conditions. Below this level, 21 slight cases were encountered against only 11 of a severe nature. These results presented mean percentile figures of 28.7 for Class I cases of neurotic tendency, and 65.8 for Class II cases. The respective medians were 12.0 and 74.0. The mean and median values for the introversion-extroversion percentiles give similarly positive results. Such findings would seem to anticipate significant results with continued study and would obviate the merits of further investigation with larger numbers and varying types of subjects.

3. Studies of Ovarian Function in Periodontosis. *Maxwell Karshan, Roslyn Wiener and Benjamin Tenenbaum, School of Dental and Oral Surgery, Columbia University, New York, N. Y.* Inasmuch as periodontosis is a disease that occurs predominantly in women during the reproductive period, studies were carried out which might throw light on ovarian function in patients with this disease. This was done by determining the estrogen content of urine, and by serial vaginal smears and basal body temperature recordings. For the estrogen determination, 24-hour urine specimens were collected generally on the seventh day of the menstrual cycle, and on the day on which there was a marked rise in temperature. Vaginal smears were made on alternate days during a complete cycle beginning with the first day. Twenty cases have been studied thus far. With 2 exceptions the cycles were ovulatory according to the indices employed, i.e., the degree of cornification, evolution of cell type and temperature. In the ovulatory cases, the cycles varied from poor to excellent. In one of the anovulatory cases, there was a deficiency of estrogen secretion according to all indices employed. With the exception of this case, the estrogen output in the urine samples collected was within the accepted normal limits.

4. A Histochemical Evaluation of Normal and Inflamed Human Gingiva. *Samuel Turesky, Irving Glickman, and Tamara Litwin, Tufts College Dental School, Boston, Mass.* A histochemical study was undertaken to evaluate the structures of normal gingiva and the changes to which they were subject in the course of chronic inflammation. The material consisted of 20 specimens of normal and inflamed human gingiva from 16 men and women ranging from 20 to 54 years of age. The tissues were examined using the Lillie and Hotchkiss leucofuchsin periodic-acid methods for the study of ground substance, basement membrane and glycogen; and the Gomori technique for the study of alkaline phosphatase. Each specimen was also examined using the hematoxylin and eosin technique, and was subjected to control study for the presence of free aldehydes, free iodates and periodates, and preformed mineral deposits. Comparison of the normal and inflamed gingivae revealed reduction in glycogen and ground substance, and thinning out of the basement membrane in chronic gingivitis. Alkaline phosphatase which was present in the endothelial cells of the scattered capillaries in the normal gingiva appeared concentrated in the inflammatory cells, fibrocytes, endothelial cells, and collagen fibers in the areas of gingival inflammation.

5. Alkaline Phosphatase Activity of Healing Gingival Wounds in the Rat. *J. R. Ring, Washington University School of Dentistry, St. Louis, Mo.* Histochemical methods demonstrate only negligible quantities of active alkaline phosphatase enzyme in the normal gingivae of the rat (and man). In order to test the hypothesis that alkaline phosphatase is associated with growth and especially with keratinization of oral epithelial membranes, clean wounds were inflicted in the gingivae of 47 normal male and female albino rats. The animals were sacrificed 24 to 72 hours after incising the interdental papilla between the upper incisor teeth. The papillae were removed and fixed in cold 80 per cent ethyl alcohol. Sections were prepared and incubated in buffered glycerophosphate substrate according to the phosphatase visualization method of Gomori. Activity of the enzyme was high in the vicinity of the healing cut. Precipitation of cobalt sulfide indicative of alkaline phosphatase activity was especially prominent in the fibroblasts and fibers, as well as leucocytes and other free cells, of the developing scar. Activity of epithelial phosphatase was highest in the superficial layers. In early stages of healing enzymatic activity was augmented in deeper layers of the epithelium as well. In most instances, healing was essentially complete by 72 hours, and epithelial phosphatase returned to the low level seen in the normal tissue.

6. The Effect of Cortisone Acetate (11-Dehydro-17 hydroxycorticosterone-21 acetate) on the Periodontal Tissues of White Mice. *Irving Glickman, Irving C. Stone, T. N. Chawla, Tufts College Dental School, Boston, Mass.* Forty male and female young littermate white mice weighing 16 to 22 grams were injected daily with Cortone Acetate* in doses of 0.5 mg., 1.0 mg., and 2.0 mg. Uninjected animals and animals injected with saline served as controls. The animals were sacrificed at intervals up to 43 days. At the time of sacrifice, the mandible, maxilla, vertebra, femur, and adrenals were removed and prepared for histological study. Microscopic examination of the periodontal structures of the animals receiving 0.5 mg. of cortisone revealed the following changes: osteoporosis of alveolar bone characterized by marked reduction in osteoblastic activity, capillary dilatation and engorgement with hemorrhage in the periodontal membrane and gingival connective tissues, reduction in the number and degeneration of the collagen fibers of the periodontal membrane. Gingival inflammation was present in both control and cortisone-treated animals associated with local irritation, but was more marked in the latter group.

7. Preliminary Report of a Study of Gingival Disease in Young Women. *Emmett R. Costich and John W. Hein. School of Medicine and Dentistry, University of Rochester and Eastman Dental Dispensary, Rochester, N. Y.* Gingival examinations were carried out on 104 student nurses ranging in age from 18 to 21 with a mean age of 20. The subjects answered questions concerning their dental habits: a) dentifrice used; b) frequency of brushing; c) length of time using dentifrice. The gingival condition of each subject was classified as normal, chronic, subacute, or acute depending on the amounts of hyperemia, edema, and proliferation present in the margins and papillae of the buccal and labial gingivae. Of the 104 girls there were: 16 normal, 15 acute, 10 chronic, and 63 subacute. Four weeks later the examinations were repeated. Of the 16 normals, 4 became subacute; of the 15 acute, 4 became subacute; of the 10 chronic, 2 became subacute; of the 63 subacute, 4 became normal, 6 chronic, and 9 acute. Thus 28 per cent of the individuals changed over a period of 30 days, 14 per cent improving and 14 per cent becoming worse. Salivary lactobacillus counts using the specific lactobacillus media of Ragosa, the volume

*A brand of cortisone acetate by Merck and Company, Inc.

of saliva secreted and the amount of debris were determined on paraffin stimulated saliva samples collected over a 5-minute period upon arising on examination days. Of 31 girls using ammoniated dentifrices from 3 months to 2 years, the lactobacillus counts and incidence of gingival disease showed the same distribution as the nonammoniated group. Volume of saliva secreted, amount of debris, and amount of debris per milliliter did not relate with lactobacillus counts. The degree of gingivitis did not relate with salivary debris.

8. Cutaneous Hypersensitivity to Procaine and Its Homologues. *Frank J. Orland, Zoller Memorial Dental Clinic and the Dept. of Bacteriology and Parasitology, University of Chicago, Chicago, Ill.* Dermal hypersensitivity to procaine is an affliction of many dental practitioners. According to a survey by the A.D.A., at least 700 dentists were reported to be subject to this unique allergic reaction. Early observation indicated this particular hypersensitivity followed the pattern of other serological reactions. Dentists with clinical histories of procaine dermatitis served to test procaine and its chemical homologues as well as structurally unrelated local anesthetics, such as Lidocaine. A 2 per cent solution of these substances was applied in drop and patch tests to the skin of such individuals. Repeated results observed in 6 different individuals clearly indicated that the skin when previously sensitized to procaine will also react with vivid erythema and edema to all compounds having in their structural formula a p-aminobenzoic acid ester, in which the side chain contains a secondary or tertiary amine-nitrogen. The length of this carbon chain may vary and the substitution of alkyl groups for hydrogen atoms in the aromatic amino group or in the side chain will not alter the antigenic effect. Substances of this group include Butyn, Monocaine, procaine, Tutocaine. Substances not eliciting a dermal reaction and not possessing the offending molecular configuration include Apothesine, Benzocaine, Lidocaine, Piperocaine (metycaine). The passive transfer test of Prausnitz-Kuestner was negative; this indicates the improbability of circulating antibodies in such cutaneous hypersensitization phenomena. It is postulated that the offending molecules constitute haptenic groups which react with antibodies fixed in dermal cells. Further study in animals is under way at present.

9. Levo-Arterenol (Levophed [R] as a Vasoconstrictor in Local Anesthetic Solutions. *Sidney Epstein, D.D.S., Albert H. Throndson, D.D.S., J. Leonard Schmitz, D.D.S., College of Physicians and Surgeons, San Francisco, Calif.* Arterenol has been known for many years but the levo isomer was isolated only recently. Toxicity figures indicate that, for equivalent pressor doses, the safety of levo-arterenol is approximately 4 times that of levo-epinephrine. Clinical studies on both operative and surgery patients reported in this study indicate that levo-arterenol used as the vasoconstrictor at a concentration of 1:30,000 gives a higher incidence of adequate anesthesia than either epinephrine 1:50,000 or nordefrin (Cobefrin [R]) 1:10,000. However, the incidence of some side-effects such as perspiration and respiratory embarrassment was also slightly higher with Levophed [R]. The solution giving the most satisfactory anesthesia was procaine 2%, tetracaine (Pontocaine [R]) 0.15 per cent, with levo arterenol (Levophed [R]) 1:30,000. The solution showing the highest degree of freedom from reactions was procaine 2 per cent, tetracaine 0.15 per cent, with nordefrin 1:10,000. It is concluded that levo-arterenol is a highly effective vasoconstrictor for dental local anesthetics which merits further clinical evaluation.

10. The Use of Intracaine Amide as a Local Anesthetic Agent. *G. W. Rapp, Loyola University School of Dentistry, Chicago, Illinois.* The pharmacology of a new drug, intracaine amide, an amide analog of the traditional ester-

type local anesthetic, has been investigated. It was found that this drug is a potent local anesthetic agent. Its activity alone compares favorably with the procaine-epinephrine combinations. It has a very low toxicity, rapid onset and moderate duration. It is not antagonistic to sulfa drugs. It is very stable in aqueous solutions. It is well tolerated by the tissues, even though there seems to be no hydrolytic enzymes in the system for this type of drug.

11. Preliminary Report on a New Local Anesthetic Agent, *Edward C. Dobbs, Department of Pharmacology and Therapeutics, Dental School, University of Maryland, Baltimore, Md.* This new anesthetic was developed at the Sterling-Winthrop Research Institute. Chemically it is an ester of para-amino-salicylic acid in which the hydroxy group in the 2 position is replaced by a propoxy. Practically, the compound is not too different from procaine. Animal experimentation suggests that it is 5 to 7 times more active, when injected, than procaine, with about half the topical activity of cocaine. The anesthetic potency is apparently potentiated by the addition of a vasoconstrictor drug. The rabbit eye test demonstrated that it is about one-fourth as irritating as tetracaine (Pontocaine [R]). Intravenous injections cause a lowering of blood pressure in barbitalized dogs, much like procaine. Studies on the intravenous toxicity in mice showed the LD_{50} dosage to be 7.3 compared to 56 for procaine making it about 8 times as toxic by this route. Our contribution in this study was to determine the efficacy and safety of this agent on patients. As operative anesthesia is more difficult to attain, this was the only type of anesthesia considered in the study. As operative procedures require considerable time, it was possible to get some data on the duration of anesthesia. Patients were given postal cards for recording the hour at which the numbness left the area, thus furnishing data on the duration of numbness. The operators were senior dental students and the subjects were patients attending the dental clinic. The operators were unaware of the contents of the prepared solutions. The following chart compares this new anesthetic (0.75 per cent, with Levophed 1/30,000) with procaine 2 per cent and epinephrine 1/50,000 as the control.

	NO. CASES	SATISF. ANES. (%)	PRODUC-TION TIME (MIN.)	DURATION		NO. REACTING (%)
				ANES. (MIN.)	NUMB (HRS.)	
New Anesthetic	35	90	1.2	50	2½	100
Control	97	72	1.8	64	3	90

It appears from this preliminary report that the new anesthetic compares favorably, if not superiorly, to the control solution. Additional data is necessary to make these figures statistically significant.

IV. Second Session: Afternoon, March 17; Abstracts 12-24.

12. Some Observations on Dental Caries in Central China. *Dimitri A. Afonsky, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* A survey of 3,349 male and female Chinese students between the ages of 12 and 27 years of age showed that 71.6 per cent were DMF-negative. Of the 95,830 permanent teeth examined, 2.46 per cent were found to have been affected by caries, or an average of 0.7 of a tooth per person. Of the persons suffering from caries 89.2 per cent had from 1 to 4 DMF teeth per mouth; 10.7 per cent had from 5 to 8; and 0.1 per cent had from 9 to 11 DMF teeth. Of all the carious teeth, 83.4 per cent had only one surface involved. The first and second molars accounted for 93.7 per cent of the DMF teeth (the mandibular first molars accounting for 45.7 per cent). Eighty-three per cent of the cavities

in the mandibular first and 93.3 per cent of the cavities in the mandibular second molars occurred in the pits and fissures of the occlusal surfaces. Caries of the anterior teeth was extremely rare. A detailed dietary survey revealed that the diet was deficient in calories and many essential nutrients, especially animal proteins, calcium, vitamin A, riboflavin, and niacin. Up to 82 per cent of the calories were derived from carbohydrates, predominantly polished rice. Sugar consumption was very low. It appeared from this survey that the nutritionally deficient Oriental diet rich in carbohydrates (rice) does not predispose to caries. (Published in *J. D. Res.* **30**: 53, 1951.)

13. A Study of Dental Caries Prevalence in School Children. *Robert J. Bruckner, Thomas J. Hill, and John Sims, School of Dentisty Western Reserve University, Cleveland, Ohio.* A dental survey was carried out on 2 groups of school children by means of mirror and explorer examination and bite-wing roentgenograms. One group consisted of 548 children, 6-16 years of age; the other of 566 children, 6-12 years old. Because of variations in tooth population between and within age groups during the years of permanent tooth eruption, the survey is submitted to show that a more accurate and meaningful measure of caries prevalence can be obtained if the ratio DMF Surfaces/Total Surfaces Available is employed, rather than DMF/Individual. "M", the number of decayed and filled surfaces on teeth missing because of caries, was determined from the number of decayed and filled surfaces on teeth which warranted extraction. For one group of children the value of M was 2.7 for posterior teeth and 1.5 for anteriors; for the other group a value of 2.5 for posterior teeth was obtained. Caries prevalence in males and females was found not to differ significantly. The tendency for girls to have a higher average DMF than boys of corresponding age was attributed to earlier tooth eruption in females rather than to inherent differences in caries susceptibilty. The influence of age distribution on the measurement of caries prevalence was demonstrated. This source of error is frequently overlooked when comparisons are made between caries attack in two or more groups of individuals.

14. Incidence of Dental Caries in Primary Teeth in Children 2-6 Years of Age, (A Preliminary Report). *Bhim Sen Savara and Theodore Suher, University of Oregon Dental School, Portland, Oregon.* Four hundred Portland children, ranging in age from 2 to 6 years were examined. They were divided into a low and a moderately high income group. The DMF Index for dental caries experience of permanent teeth (Klein and Palmer, 1938) was modified slightly and used for recording the caries experience of primary teeth. The analysis of the data revealed that there was a definite difference between the two groups with regard to caries prevalence and experience. In the high income group, 57.5 per cent of the children had caries as compared to 76 per cent of the children in the low income group. A marked difference in dental caries experience was observed between these groups at each age level. For example, the maximum difference was at 4 years of age, when the children from the high income group had a DMF Index of 2.4 teeth as compared to the other group which had a DMF Index of 4.7 teeth. This difference between the two groups of children was also present and maintained at each age level with regard to DMF tooth surfaces. The children of the high income level showed, on the average, four tooth surfaces less involved than the children of the corresponding ages in the low income group. The ratio of the DMF teeth to DMF tooth surfaces indicated that one surface per tooth was affected at 2 and 3 years of age. This ratio increased in the low income group to 2:1 at 4 years so that two surface involvements occurred at and after 4 years. In the high income group, the same experience appeared at 5 years of age and after.

15. Caries Incidence in Dental Students. *B. W. Warner, T. J. Howard, and H. I. Myers, School of Dentistry, The University of Kansas City, Kansas City, Mo.* Approximately 100 dental students (freshmen) were given complete roentgenograms and a complete oral examination. Subsequently acidophilic counts were made on each student. All of the decayed teeth, missing teeth, filled teeth (filled minus decay) and total cavities (all fillings and decay on all surfaces) were counted and tabulated. One year later this entire class was reexamined using the same techniques. The DMF and total cavitation was tabulated as above and all new decay was counted on all surfaces. The mean was determined in the 5 plus group (5 new cavities or more since last year) and the average acidophilic count for the group was run. The group having no new decay, since last year, and their respective group acidophilic count mean was compared statistically with the former group. Correlations were noted as follows: (a) the group having the greatest acidophilic count was the group having the highest caries incidence over the year. (b) The group having no new decay over the year had the lowest acidophilic count.

16. The Variability of Progression of Dental Caries on the Occlusal Surfaces of Second Permanent Molar Teeth. *Julian D. Boyd, M.D., Department of Pediatrics, State University of Iowa, Iowa City.* The four-year serial dental records of approximately 200 teen-aged inmates of a custodial institution have been reviewed, with particular attention to the rate, pattern and regularity of advance of new cavities appearing on the occlusal surfaces of permanent second molar teeth. The subjects had had minimal reparative dental service prior to the study, and all local repair or therapy was avoided as a routine throughout the course of the long periods of observation. The data are presented in terms of the frequency distribution of the respective findings, as well as in terms of average findings. This presentation is one of a series of studies dealing with the individual and secular variability of caries advance under grossly similar conditions of living and of diet.

17. Unfavorable Report From Kansas Community Using Artificial Fluoridation of City Water Supply for Three-Year Period. *Charles A. Scrivener, College of Physicians and Surgeons, School of Dentistry, San Francisco, Calif.* The recent experiences in the addition of fluorides to community water supplies, which have resulted in its almost universal acceptance as an effective dental caries preventive measure, are not shared by a Kansas town. In 1946 the Kansas State Board of Health selected Ottawa, Kansas, as the site for a pilot study. Seven-tenths of one p.p.m. sodium fluoride solution was added to the water to bring the concentration to 1 p.p.m. F after it has passed through the softening process. Daily tests have been made at the local plant and twice weekly samples have been sent to the state laboratory in order to assure constant and uniform concentration. A carefully conducted dental examination at the beginning of the test period in 1946 disclosed the following data for approximately 100 children, 5 to 6 years of age: Average teeth ABC (DMF) per child. .250. Caries-free children, 82.3 per cent. In 1949 a routine examination of the same age group, the same number of children disclosed: Average teeth ABC (DMF) per child, .670. Caries-free children, 45.0 per cent. This report is offered merely to demonstrate that the results of the fluoride experiment are not universally favorable. No explanation is attempted and the test will continue to run its 10-year trial period.

18. The Evanston Dental Caries Study VII. The Effect of Artificially Fluoridated Water on Dental Caries Experience. *I. N. Hill, J. Roy Blayney and Walter Wolf, Zoller Memorial Dental Clinic, University of Chicago, Chicago, Ill.* Previous reports of the Evanston Dental Caries Study have indicated no

reduction attributed to fluorine in the caries experience of the deciduous teeth of the 6-, 7- and 8-year-old children. This is thought to be due to the fact that all of the deciduous teeth were developed and were present in the mouth for some time prior to the introduction of sodium fluoride in the communal water supply. The recent analysis of the data collected from the 12-, 13- and 14-year old children indicates a reduction in the DMF permanent teeth of approximately 12.19 per cent. The carious lesions of the permanent teeth were reduced by 55.7 per cent for the 12-year-olds, 38.80 per cent for the 13-year-olds and 33.3 per cent for the 14-year-olds. The total number of children examined (1,769) for this age group indicated a 73.21 per cent increase of those who are immune clinically and radiographically to dental caries. (To be published in *J. D. Res.*)

19. The Evanston Dental Caries Study. VIII. Fluorine Content of Vegetables Cooked in Fluorine-Containing Waters. *Donald J. Martin, Evanston Health Department, Evanston, Ill.* The fluorine content of vegetables was studied for the purpose of providing information regarding the probable amount of fluorine injested in the daily diet. A questionaire was sent to 15 grocery stores, representing all sections of the city, to determine which types of vegetables (fresh, frozen, or canned) sell in greatest quantity, and which kinds of vegetables in each category are in greatest demand. The fresh and frozen vegetables, all secured upon the open market in Evanston, were analyzed in both the raw and the cooked state. To simulate methods of cooking in the home, duplicate batches of the vegetables were cooked in a saucepan and in a pressure cooker. Water containing 0.0, 1.0, 2.0, and 5.0 parts per million of fluorine was used in determining the amount of fluorine absorbed by the vegetables in the cooking process. Analyses of the 9 different vegetables studied showed that they do absorb and retain, in varying amounts, some of the fluorine from the water in which they are cooked. These analyses also revealed that the vegetables cooked in a saucepan absorbed more fluorine than those prepared in a pressure cooker.

20. Preliminary Investigations of the Effect of Sodium Monofluorophosphate on Salivary Acid Production and Hydroxy Apatite Solubility. *Glen B. Haydon, John W. Hein, and Dwight E. Gardner. School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* The equal caries inhibiting potential and lower toxicity of fluoride as Na_2FPO_3 as compared to NaF was reported by Shourie and associates (*J. D. Res., August, 1950*). In the present investigation it was found that fluoride as Na_2FPO_3 was approximately twice as effective as fluoride as NaF in inhibiting acid production in saliva-glucose mixtures. It was also shown that while increasing amounts of calcium ions progressively decreased the effectiveness of NaF against salivary acid production, no such decrease was observed for Na_2FPO_3. While the calcium salt of H_2FPO_3 is soluble to the extent of 1 Gm. in 100 ml. of water, it was found, however, that Na_2FPO_3 decreased the solubility of hydroxy apatite. Samples of hydroxy apatite were treated with Na_2FPO_3 and NaF at levels of 20, 40, 80, 160, and 320 ppm fluoride. The apatite treated with Na_2FPO_3 at the lower levels of fluoride showed a greater solubility than that treated with NaF. At the higher levels of fluoride the situation was reversed. Fluorine analyses of NaF treated apatite showed an increase to a saturation between the 80 and 160 ppm levels, while the fluorine content of Na_2FPO_3 treated apatite increased at each successive level. The fluorine content of Na_2FPO_3 treated apatite was always below the NaF, being about ½ at the 80 ppm level.

21. Further Studies of the Caries Inhibitory Potential and Acute Toxicity of Complex Fluorides. *John W. Hein, Frank A. Smith, Dwight E. Gardner, William L. Downs, Elliot A. Maynard, and Harold C. Hodge. School*

of Medicine and Dentistry, University of Rochester, Rochester, N. Y. Drinking solutions containing synthetic Na_3AlF_6 (19.5 ppm F), Flural (Al, F, SO_4, 16 ppm F), KPF_6 (40 ppm and 114 ppm F), Na_2FPO_3 (20 ppm F), and NaF (17.3, 12.3, and 8.9 ppm F) were administered to weanling Syrian hamsters on a caries-producing diet. A nearly maximal, statistically significant, reduction in the Total Caries Scores as compared to littermated controls were obtained with the N_3AlF_6, Flural, Na_2FPO_3, and NaF (17.3 and 12.3 ppm F). Neither level of KPF_6 gave a statistically significant reduction in caries scores. Sodium fluoride at 8.9 ppm F gave an 80 per cent reduction in caries score, but this was not statistically different from the 94 per cent reduction obtained with 12.3 ppm F as NaF. The 24-hour intraperitoneal acute toxicity of the various fluorides as compared to NaF, on a fluoride content basis, was: Na_3AlF_6, .007 as toxic; Flural, .6 to .5 as toxic; KPF_6, .1 as toxic; and Na_2FPO_3, .4 to .3 as toxic. The fluoride content of the erupted half of the lower incisors of the male animals on the basis of ashed weight was proportional to the F level of the drinking solutions for NaF, but was less than predicted for Flural, KPF_6, and Na_3AlF_6. The F content of the tibias of the NaF, Na_3AlF_6, and Flural males was proportional to the F content of the drinking solutions.

22. Relation of Specific Surface Area of Powdered Human Enamel to the Adsorption of Fluorine. *R. C. Likins, D. B. Cowie and F. J. McClure, National Institute of Dental Health, Bethesda, Md.* Human enamel separated from nonfluorosed teeth by the Manly-Hodge technique was fractionated between 60-120 and 200-325 mesh screens and the specific surface area of each determined by nitrogen adsorption (2.1 m^2/Gm. and 3.7 m^2/Gm. respectively). Samples aliquots of the sieve fractions were then shaken for 30 minutes at 20° C. in each of 4 NaF solution (0.0001, 0.001, 0.01 and 0.1 mole of F per liter), containing added radioactive F^{18}. The suspensions were immediately filtered, dried at 105° C., and the amount of fluorine in the treated samples determined by radioactivity measurement. The results indicate that fluorine is adsorbed by powdered human enamel in proportion to the specific surface area as determined by nitrogen adsorption.

23. Fluoride Exchange With Hydroxyl as the Mechanism of Fixation of Fluoride by Bone. *David Megirian and Harold C. Hodge. School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* There has been a continuing argument as to whether fluoride replaces phosphate in bone or tooth mineral to form calcium fluoride. Neuman and associates recently described experiments in which fluoride exchanges with hydroxyl presumably to form fluorapatite. This work has been confirmed in the present report. Twelve milligram samples of the glycol ash of rabbit bone powder have been placed in contact with 120 ml. of a phosphate buffer solution containing 10 micrograms per milliliter of fluoride at pHs ranging from 6 to 10. The suspension was stirred for 4 days. The bone powders were pretreated at 37° C. with phosphate. With increasing pH less and less fluoride appeared on the bone. The results may be simply explained by assuming competition between hydroxyl and fluoride ions for positions on the surfaces of the microcrystals of hydroxylapatite to form the fluorapatite lattice.

24. Fluoride Turnover in the Growing Skeleton. *William B. Savchuck and Wallace D. Armstrong, Dept. of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.* Studies on a nontoxic level of fluoride intake (20 ppm in drinking water) in 3 colonies of young or mature animals comprising 232 male rats were based on microdeterminations of fluoride in humeri. Fluoride analyses of the various skeletal components suggest the use of the humerus as a

typical bone. Uptake of fluoride on a 60-day fluoride regimen was at a logarithmic rate. Upon withdrawal of fluoride supplement, elimination of fluoride from the humerus was limited in degree. Expressing analytical results in terms of micrograms rather than per cent indicates no appreciable decrement in total fluoride between the twentieth and one hundred fiftieth postfluoride days. The persistently growing incisors exhibited significantly higher fluoride contents after 150 days of elimination as compared with controls, despite an extremely low (0.6 ppm) fluoride ration. This is evidence of the skeletal flux whereby stored fluoride is almost imperceptibly released elsewhere and made available in a utilizable form for anabolic processes. In the uptake of fluoride, dentin was more responsive than enamel during both the fluoride regimen and the postfluoride period.

V. Third Session: Section A, Evening, March 17; Abstracts 25-36.

25. The Occurrence of Yeasts in the Saliva of Children. *Robert L. Glass, Tufts College Dental School, Boston, Mass.* Stimulated saliva samples were collected from 348 children, 7 to 14 years of age, whose caries activity during the past year was known. One half cubic centimeter portions were plated on Sabouraud's dextrose agar; colony counts were made after 48 hours incubation at 37° C. Yeasts were found in 156 cases, a 45 per cent occurrence, in counts up to 12,000 per cubic centimeter of saliva. *Candida albicans* predominated; Cryptococci and Saccharomyces strains were found occasionally. There was no correlation between yeasts and lactobacilli counts. A significant correlation was found between the presence of yeasts and the incidence of dental caries.

26. The Frequency of Occurrence, and Saliva Compatibility of Organisms from Saliva Antagonistic to some Lactobacilli. *C. A. Scrivener, B. W. Warner, H. I. Myers, and N. A. Moore, School of Dentistry, University of Kansas City, Kansas City, Mo.* Early morning saliva from 98 dental students was collected according to the standard lactobacillus count technique. One-tenth c.c. of a 1:100, 1:1000, and 1:10,000 dilution of the thoroughly shaken saliva was pour plated in blood agar base medium. The plates were aerobically incubated 72 hours at 37° C. At this time the growth on these plates was covered with Kulp's tomato juice agar pH 6.1 heavily innoculated with Hadley lactobacillus (A.T.C.#4646). The plates were reincubated aerobically at 37° C. for 96 hours. The plates were then examined on a Quebec colony counter. In some instances, over the saliva bacterial colonies a clear halo of complete inhibition of lactobacillus growth was observed. In others, there was a ring of larger lactobacillus colonies resembling satellitism. In a few cases, a zone of large lactobacillus colonies at the complete inhibition halo periphery was observed. All the complete inhibition types that could be subcultured and representative types of the satellitism producing saliva colonies were established in pure culture, classified, tested for inhibition by cross streaking with three lactobacillus types, and tested for saliva compatibility with the agar well technique. Twenty-five of the 98 salivas showed some complete inhibition. Of these, 24 also showed seeming satellitism. In all, 89 salivas showed this latter phenomenon, and only 8 showed neither phenomenon. The number of organisms, representing 18 or more species, showing these 2 phenomena ranged from zero to 3,700,000 per c.c. saliva. Of the 25 pure cultures tested by cross-streaking, 19 showed inhibition, but no evidence of satellitism or stimulation was found. Of the 25 pure cultures tested against 16 salivas with the well tecnique, 16 showed excellent compatibility and 4, poor compatibility. Five pure cultures gave insufficient growth for evaluation.

27. Comparative Cultural Characteristics of Oral Actinomycetes, Lactobacilli and Streptococci. *Valerie Hurst, Patricia Mullett, Richard Gaard and James Nuckolls, University of California College of Dentistry, San Francisco,*

Calif. Actinomycetes, lactobacilli and alpha streptococci are considered associated with the carious lesion and have been found to be potentially cariogenic in vitro. Since all 3 of these groups, particularly the actinomycetes and lactobacilli, are highly variable in morphology, a study of their distinctive cultural characteristics may be of value. In preliminary studies, 5 strains of lactobacilli were found to grow on media adjusted to pH 4 or lower, while 10 strains of actinomycetes did not grow below pH 5. Lactobacilli did not grow at initial pH values above 8.4 whereas 9 actinomycetes grew at initial pH values below 9.0 and 9.5 and one strain below pH 10. Two strains of alpha streptococci and one strain of *Streptococcus salivarius* grew at pH 9.7. Because of the reported value of bile tolerance in classifying streptococci, a comparison was made of the inhibiting concentrations of bile for all 3 groups. Five lactobacilli were able to grow on media containing 40 per cent bile, while 6 actinomycetes were completely inhibited with 2 per cent bile and did not grow well with concentrations much above 0.1 per cent. *Str. salivarius* and one alpha streptococcus were inhibited by 2 per cent while the other was inhibited by 5 per cent bile. The actinomycetes failed to grow on media containing 0.008 per cent crystal violet, while the lactobacilli and streptococci grew well with this concentration. In general, little difference was found in the concentrations of methylene blue, potassium tellurite, or sodium chloride tolerated by these bacteria.

28. The Effect of Water Soluble Chlorophyll on Pure Cultures of Organisms Commonly Found in the Oral Cavity. *Thomas A. Nevin and Basil G. Bibby, Eastman Dental Dispensary, Rochester, N. Y.* Fifteen strains of organisms, including 5 members of each of the following genera: lactobacillus, streptococcus, micrococcus, as well as pooled saliva, when feasible, were studied in an effort to determine how they would react to various concentrations of water soluble chlorophyll. ²Two strains of each genus were obtained from the American Type Culture Collection. ³The rest were isolated from patients at the Eastman Dental Dispensary. ⁴Standard testing methods were used throughout the experiment. ⁵In all, 3 series of experiments were carried out. ⁶The first, to demonstrate whether chlorophyll had an inhibitory effect on the organisms used, and to indicate the nature of this effect; the second, to determine whether there would be any interference in the glycolytic scheme; the third, to determine if there would be any interference in the respiratory habits of those organisms with which measurements of this type could be made. ⁷The evidence presented here shows that water soluble chlorophyll exerts a bacteriostatic effect on all of the organisms studied; that the inhibition of respiration is roughly proportional to the concentration of chlorophyll; and that the glycolytic activity of the individual cell may be stimulated.

29. Effects of Various Porphyrin Compounds Observed on Pure Cultures of Six Species of the Family Lactobacteriaceae. *Wm. David McBride, University of Minnesota, Minneapolis, Minn.* Previous reports on the efficacy of chlorophyllin compounds for inhibiting dental caries prompted this study. A technique whereby samples of material could be removed from pure cultures periodically for the determination of acid production was devised. Preliminary results showed autoclaving of sodium-copper-chlorophyllin for 15 minutes greatly decreased its inhibitory activity for *Lactobacillus casei*. Unautoclaved, sodium-copper-chlorophyllin inhibited acid production by *L. casei, L. delbruckii,* and a Group F Streptococcus, whereas it stimulated acid production by *L. arabinosus, L. leishmannii,* and Leuconostoc mesenteroides. Sodium-magnesium-chlorophyllin had essentially the same action except that an optimum concentration rather than a directly proportional concentration showed maximum effectiveness in several cases of inhibition. Iron protoporphyrin chloride, free protopor-

phyrin, hematoporphyrin hydrochloride, and bilirubin showed inhibitory activity for *L. casei*. It is therefore clear that the inhibition of acid production by the chlorophyllins is not a specific activity, but rather a general property of several porphyrin compounds for certain types of organisms whereas other types of acidogenic organisms are stimulated by these compounds.

30. The Role of Antibiotics and Enzyme Inhibitors in Relation to Lactobacillus Counts II. *W. E. Ludwick, Dental Research Facility, Naval Training Center, Great Lakes, Ill., and L. S. Fosdick, Northwestern University Dental School, Chicago, Ill.* The lactobacillus count was adopted as a criteria for measuring the effectiveness of 12 dentifrices modified with an antibiotic or an enzyme inhibitor. Groups of 65 recruits each were selected to test the dentifrices for a 7- to 9-week period. Four other groups served as controls for the study. The dentifrices were modified with aureomycin, chlorinated phenol, chloromycetin, copper chlorophyllin, penicillin or white dye. Comparison of the pretreatment with post-treatment median lactobacillus counts indicate a range of 65 per cent decrease to an increase of 23 per cent, while the control groups ranged from a 66 per cent decrease to a 17 per cent increase.

31. Influence of Ammonia and Urea Upon Lactobacillus Acidophilus 4646. *Sholom Pearlman, John A. Muntz and Thomas J. Hill, Institute of Pathology, School of Dentistry, and School of Medicine, Western Reserve University, Cleveland, Ohio.* The inhibitory effect of ammonium phosphate upon the growth of *L. acidophilus 4646*, and the ability of potassium to negate this effect, have been confirmed by the use of "synthetic" broth at alkaline pH (7.5). Inhibition occurred only when the cells were present initially in very small numbers. Growth was inhibited completely in 6 per cent urea, but only moderately in 3 per cent urea, at pH 7.5. In the presence of either potassium or ammonium phosphate (5 per cent), urea produced only moderate additional inhibition in concentrations of less than 3 per cent. Glycolytic activity of washed whole-cell preparations was studied at pH 7.4, in the Warburg respirometer. Acid production was inhibited completely by 24 per cent urea, and only partially by lesser concentrations; at the 6 per cent level inhibition was of questionable intensity. Preincubation of the cells with 24 per cent urea for 30 minutes or more resulted in significant reduction of their glycolytic activity. Urea was not hydrolyzed to ammonia during these experiments. In assessing the possible value of these compounds in controlling oral lactobacilli, consideration should be given to the availability of potassium in saliva and to the high concentrations and alkalinity which must be sustained to produce inhibitory effects in vitro. (Published in *J. D. Res.* **30**: 542, 1951, this issue.)

32. Effect of Graded Amounts of Vitamins on Acid Production by Oral Lactobacilli. *Stewart A. Koser, Barbara Jane Fisher, and Shirley Kauffman, Walter G. Zoller Memorial Dental Clinic and the Department of Bacteriology and Parasitology, University of Chicago, Chicago, Ill.* Both pH and titratable acid produced by representative oral lactobacilli were determined in a basal medium of hydrolyzed casein, glucose, and salts supplemented with varying amounts of required vitamins. The amounts ranged from distinctly suboptimum to quantities sufficient to support maximal growth. In the presence of smaller quantities of a needed vitamin growth acid production was limited, the limitation being in general proportional to the amount of vitamin supplied. The following amounts of each vitamin were sufficient to support maximal, or practically maximal growth, and acid production under the conditions of these tests: in terms of micrograms per milliliter of medium, riboflavin 0.03, nicotinic acid 0.05, calcium pantothenate 0.03 to 0.1, biotin 0.0003, pteroyl-

glutamic acid (folic acid) 0.01, pyridoxamine dihydrochloride 0.3-1.0, thiamine hydrochloride 0.01-0.03. However, amounts distinctly less than the foregoing were sufficient for production of acid between pH 5.0 and 4.0. A comparison of our data with the rather scanty information available concerning the vitamin content of saliva shows that in many instances there is sufficient vitamin in saliva to support maximal, or near maximal growth, and acid production of oral lactobacilli. (Published in *J. D. Res.* **30**: 532, this issue.)

33. Preparation of a Glycolytic Enzyme Extract From Lactobacillus Acidophilus 4646. *Robert E. Gillis, Department of Microbiology, Western Reserve University, Cleveland, Ohio.* It is known that with many bacteria the dissimilation of glucose follows the same general pattern as has been demonstrated for various mammalian tissues and yeasts, namely a process of phosphorylytic steps resulting in the formation of lactic acid. These systems are best elucidated by the use of nonproliferating cells and cell-free extracts. In order to obtain large amounts of cells for glycolytic experiments, it was necessary to develop cultural conditions which would yield abundant growth. A basal medium consisting of Difco yeast extract 0.5 per cent, glucose 1 per cent, potassium monobasic phosphate 0.5 per cent, sodium acetate 1 per cent, distilled water 80 per cent, tap water 20 per cent, was found to give an average yield of 1 to 1.5 Gm. (wet weight) of cells per liter of medium. By the addition of tomato juice filtrate and a mixture of inorganic salts to the above basal medium an average yield of 5 to 6 Gm. (wet weight) per liter of medium was obtained. This represents a four-fold increase in yield and provides a sufficient bulk of cells for glycolytic experimentation. Nonproliferating cells of *Lactobacillus acidophilus 4646* grown in this medium were tested for their ability to produce acid from glucose in the presence of a phosphate bicarbonate buffer at 37° C. Glycolysis occurred at a rapid rate and lactic acid was quantitatively produced. Cell-free extracts were then prepared by fractionating the cells suspended in phosphate bicarbonate buffer in the sonic oscillator. These extracts were active in producing lactic acid from glucose. Work is now in progress to determine if these extracts contain the enzymes of the Embden-Meyerhof scheme.

34. Localization of Nucleic Acids and the Mechanism of the Gram Reaction in Lactobacilli. *Heiner Hoffman, Dental Research Laboratory, The Ohio State University, Columbus, Ohio.* Young lactobacilli stained with dilute basic dyes revealed large basophilic polar areas, but no basophilic layer at the cytoplasmic membrane as in the Gram reaction. A Gram-stained preparation decolorized with acid alcohol and then restained with dilute basic dye showed that the basophilia was now distributed as in the Gram reaction. If the iodine "mordant" was omitted in this last experiment, the original distribution of basophilia in simply stained cells was found. The KI and pH of the iodine solution had significant effects. Ribonuclease digestion for 2 hours revealed gram-positive granules. With methyl green-pyronin staining and 1.0 M saline extraction, it was shown that in the center of the intracellular basophilic areas is a dense body consisting of a desoxypentose nucleoprotein medulla and a pentose nucleoprotein cortex. Conclusions: The gram complex consists of 2 components differing in mobility. Localization of the gram complex at the cytoplasmic membrane results from the Gram technique itself and does not occur naturally. The second step in the Gram technique is responsible for shifting the localization of the pentose nucleic acids. The pentose nucleic acids of the gram complex surround the central body much like those about the nucleus of metabolically active higher cells, but it is possible that the bacterial central body is more than a nucleus.

35. Comparison of Three Bacteriologic Procedures for Evaluating the Significance of Lactobacillus Counts. *D. H. Rizzo and E. B. Tilden, Northwestern University Dental School, Chicago, Ill.* A group of orphanage children, the majority 3 to 11 years old, were followed by annual clinical and bacteriologic examinations made concurrently, 87 children for 1 year, and 54 for 2 years. The figures for the standard tomato agar count, the Snyder test, and the acidogenic count introduced by Davies, Slack and Tilden were compared statistically with the progress of caries, as indicated by the number of new cavities revealed by clinical and radiographic examination. The findings do not support the conclusion of those investigators who believe there is no significant association between lactobacilli and dental caries.

36. A Selective Medium for the Isolation and Enumeration of Oral Lactobacilli. *Morrison Rogosa, Joyce A. Mitchell, and Ralph F. Wiseman, National Institute of Dental Research, National Institutes of Health, Bethesda, Md.* As the result of an investigation with 2000 human salivary specimens and hamster oral samples a new, easily prepared, inexpensive and reproducible medium (SL Agar) was developed for the selective isolation and enumeration of oral lactobacilli. The composition of SL Agar is as follows:

Trypticase	10 Gm.	Yeast Extract	5 Gm.
KH_2PO_4	6 Gm.	Ammonium Citrate	2 Gm.
Salt Solution*	5 ml.	Glucose	20 Gm.
Sorbitan Monooleate	1 Gm.	Sodium Acetate	25 Gm.
Acetic Acid	1.32 ml.	Agar	15 Gm.

Distilled Water to 1 Liter, pH 5.4

*Salt Solution: $MgSO_4.7H_2O$, 11.5 Gm.; $MnSO_4.2H_2O$ 2.4 Gm. *or* $MnSO_4.4H_2O$, 2.86 Gm.; $FeSO_4.7H_2O$, 068 Gm.; distilled water to 100 ml.

SL Agar was also found to be satisfactory for the isolation of lactobacilli from the feces of hamsters. In the comparative study of 122 human salivary specimens and 156 oral hamster samples in the new SL Agar and on the Jay and Arnold modification of Hadley TJ Agar, it was found that SL Agar was almost completely specific for lactobacilli and produced counts which compared favorably with those obtained from TJ Agar. Except for the very infrequent presence of yeasts in greatly reduced numbers, all of the organisms other than lactobacilli found on TJ Agar, such as micrococci or staphylococci, streptococci, molds, spore-forming bacteria, and gram-negative rods, were eliminated in SL Agar.

VI. Third Session, Section B, Evening, March 17, Abstracts 37-48

37. Demineralization of Hard Tissues by Organic Chelating Agents. *Gordon Nikiforuk, Department of Pedodontics, Faculty of Dentistry, University of Toronto, and Leo M. Sreebny, Division of Oral Pathology, College of Dentistry, University of Illinois, Chicago, Ill.* Histological study of bone and other hard tissues has hitherto only been accomplished in acid media. The denaturation of proteins at these low pH's has limited histochemical studies of these tissues. Recently, the introduction of organic chelating agents has offered possibilities of demineralizing these tissues at neutral and alkaline pH's. The sodium salt of ethylene diamine tetracetic acid has the ability to form soluble nonionic chelates with a large number of metallic ions including calcium. Preliminary investigations with this compound showed that demineralization of bone and teeth can be accomplished at pH's ranging from 5.0 to 10.3. The time required is about the same as that needed for a comparable specimen in the formic-citric acid method, and slightly longer than that required for 5

per cent nitric acid. Preservation of structural detail and of staining properties of the tissues seems excellent. Further experiments are in progress to determine the optimal pH and concentration of the chelating compound for the preservation of enzymes and tissue elements.

38. An Intra-Oral Method for Determining the Radiopacity of Oral Structures. *Arthur H. Wuehrmann and William A. Curby, Tufts College Dental School, Boston, Mass.* The purpose of this study was to test the applicability of the high kilovoltage technique for dental radiography and to determine the optimum kilovoltage for the production of dental radiographs that are capable of demonstrating both hard and soft tissue lesions with one exposure. To accomplish this purpose it was necessary to determine the radiopacity of oral structures varying from soft tissue to enamel. An ivory intra-oral step tablet was designed to compare the radiopacity of oral structures with ivory ranging in thickness from 4.5 to 19.5 mm. The step tablet was fastened to the face of the intra-oral film and placed on the occlusal surface of the teeth. In this manner the teeth and step tablet were exposed simultaneously. Forty films were taken at different kilovoltages on 9 patients. The density of the tissues as registered on the intra-oral films varied beyond both the upper and lower ranges of the step tablet. The step tablet was exposed extra-orally at different kilovoltages for a total of 42 exposures at different milliampere second values. The findings indicate that the full range of ivory is best demonstrated at tube potentials of 90 to 95 kvp.

39. The Condition of Enamel Walls When Prepared With Different Instruments. *Edmund V. Street, College of Dentistry, University of California, San Francisco, Calif.* A laboratory study was made to determine the effects produced upon the enamel walls of extracted teeth when cut with various types of burs, stones, disks and chisels. One hundred thirteen teeth were cut longitudinally. The cut surfaces were ground and made smooth. Rotational speeds of the motor-driven instruments were varied and most of the operations were performed under both dry and wet conditions. To show the width of the scratches produced by the instrument, each section was coated with a paste of lamp black and alcohol. When dry, the excess was wiped off, leaving a residue of the paste in the scratches and grooves made by the instrument. All sections were viewed microscopically under different magnifications and photographs were made with a total magnification of 37 power. The maintenance of wet or dry conditions in the finishing of the enamel surfaces with burs or disks offered negligible differences in the specimens. Likewise, the speed of rotation did not appreciably vary the appearance. The sandpaper disk produced the smoothest finished surface. The fissure burs did not nick the enamel nearly as much as the mounted carborundum stones. The grooves left by the carborundum and diamond disks were quite prominent. Indentations, made when sharp chisels were used, do not support the hypothesis that a smooth surface on the enamel wall of a cavity can be produced by such instruments.

40. Preliminary Report on the Absorption of Radiocalcium at the Margins of Various Filling Materials Used in Restorative Dentistry. *W. J. Simon, and W. D. Armstrong, School of Dentistry and Department of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.* Buccal, Class V, cavities were prepared in 8 wet, sound, extracted, lower second premolars. Each cavity was filled; one with amalgam, one with a gold inlay, one with a gold foil, one with an oxyphosphate of zinc cement, one with silicate, and

three with acrylic resin. In three teeth filled with acrylic resin, one was filled with the resin after it had been mixed on a slab, one after polymerization had taken place for 30 seconds in a sealed jar but not spatulated, and one after polymerization had taken place for 30 seconds in a sealed jar and spatulated. After the fillings were inserted, the roots were coated with wax and the clinical crowns were immersed in a solution of radiocalcium (Ca^{45}) for 48 hours. The teeth were then washed with distilled water and embedded in methyl methacrylate resin. Horizontal cross sections were prepared through the widest gingivo-occlusal portion of the restorations with a band saw. Radioautographs were prepared on Agfa no screen film. A control, to determine the chance of technical contamination error, was run by immersing a wet, sound, extracted tooth in radiocalcium solution and then preparing and filling the cavity with acrylic resin. The radioautographs demonstrate the following: (1) The control sample reveals that the technical possibility of errors of contamination are minimal. (2) The radiocalcium penetrated the margins of all of the filling materials used but in varying amounts.

41. The Influence of the Changing Dentition on Masticatory Function. *F. R. Shiere and R. S. Manly, Tufts College Dental School, Boston, Mass.* A study was made of 65 children, whose ages ranged from 5 to 10 years, in order to compare the masticatory function of the changing dentition with the adult dentition. Performance and food platform areas were determined by the Manly-Yurkstas methods, while the maximum biting forces were measured using the Howell-Manly electronic force meter. The performance tests showed a definite increase with increase in age, regardless of sex. When the swallowing threshold was compared to performance, the children that demonstrated a higher performance tended to swallow finer particles of food. Children who showed a lower performance compensated for this deficiency by swallowing larger particles. When compared with adults, the performance of the children was 78.4 per cent as great, their swallowing threshold was 79.4 per cent as great, the area was 51.4 per cent as great, while the forces were only 33.3 per cent as great. Children tended to have a better performance with less area than adults.

42. Masticatory Function of Children With Malocclusion. *R. S. Manly, and F. S. Hoffmeistr, Laboratory for Oral Physiology and Orthodontic Department, Tufts College Dental School, Boston, Mass.* Eighty children with malocclusions were given dental orthodontic, and craniometric examinations, including models and intraoral, extraoral, and cephalometric roentgenograms. Maximum biting forces were recorded for each tooth, food platform areas were measured and masticatory performances were determined for each side of the mouth with peanuts and carrots as test foods. Masticatory performances of Class I and II cases were similar, but was lower for cusp-to-cusp cases by a significant amount. No significant relation could be found between performance and the Frankfort-Mandibular or nasion angles. The performance was related to food platform area in the same manner as has been shown for adults (Yurkstas and Manly, *J. Applied Physiol.*, July, 1950) except that both area and performance were lower among the orthodontic cases. Maximum molar forces were similar for Class I and cusp-to-cusp cases, and lower for Class II cases. Molar and incisor forces were both directly related to the size of the Frankfort-Mandibular angle, but not to nasion angle.

43. Compensation for Inadequate Mastication. *A. Yurkstas, Laboratory for Oral Physiology, Tufts College Dental School, Boston, Mass.* One hundred

and four young adults have been tested for chewing ability by a previously described volumetric method (*J. Applied Physiol.* **3**: 45, 1950). The extent of pulverization at the swallowing point and the number of strokes necessary to reach this "swallowing threshold" have also been recorded. It was found that masticatory performance was directly related to swallowing thresholds and that as masticatory capacity decreased the size of the particles in a bolus of food deemed ready for swallowing increased. A correlation coefficient of +0.702 significant to the 1 per cent level was found. No significant relationship existed between the number of strokes used to reach the "swallowing threshold" and the masticatory performance. The data indicated that individuals with a decreased masticatory capacity did not compensate for their deficiency by chewing their food for a longer period of time, but swallowed larger particles of food than persons with an adequate dentition.

44. Relation of Food Platform Area to Masticatory Performance and Efficiency. *R. S. Manly, Laboratory for Oral Physiology, Tufts College Dental School, Boston, Mass.* The masticatory performance was determined for approximately 200 half dentitions with both carrots and peanuts as test foods. The performances were compared with the food platform area, tooth units in occlusion and mesiodistal length of the molar imprints on the same subjects. The performance with peanuts was related to the 3 other measurements. The tooth units were least related and had poorest value for predicting performance. The molar imprint (MIL) was moderately correlated but was not the fundamental variable since half dentitions having essentially the same MIL still showed a correlation between area and performance, while half dentitions with constant area showed poor correlation between MIL and performance. The relationship between area and performance was not linear, but became linear when the data were plotted on logarithmic probability paper. Masticatory efficiency (20/chews required to cause 78 per cent of food to pass 10 mesh screen) was directly related to food platform area.

45. A Functional Evaluation of Fixed and Removable Bridgework. *H. H. Fridley, Dental Corps, U.S.N., and A. Yurkstas, Tufts College Dental School, Boston, Mass.* The functional merits of removable and fixed bridgework have been evaluated. Unilateral removable partials and fixed bridges have been constructed for each of 20 subjects. Auto controls were utilized. Approximately 1,600 masticatory performance determinations were carried out during the study. From a functional standpoint, there was no essential difference between fixed and removable bridgework. In both instances there was an increase of approximately 29 per cent in masticatory performance. Subjects wearing unilateral replacements, of the type studied, showed a 28 per cent increase in the degree of pulverization at the swallowing point with a 20 per cent reduction in the number of strokes required and a 30 per cent reduction in time when compared to the same side without any prosthetic replacements.

46. An Antibacterial Root Canal Cement. *Morris Cohen, and Samuel Luks, College of Dentistry, New York University, New York, N. Y.* One of the basic requirements of a root canal filling cement is that the material should be bacteriocidal or bacteriostatic in action. It should not be injurious on contact with the periapical tissues, but rather should hasten healing and prevent infection or reinfection of the treated tooth. Many root filling pastes contain chemotherapeutic agents to make the mass antibacterial, but the required concentration of the agent to be effective against microorganisms would at the same time be highly irritating to the tissues, with accompanying symptoms of

tissue irritation, inflammation and breakdown. The advent of the antibiotics, with their great range of effectiveness against micro-organisms has opened new fields for investigation in pulp canal therapy. A suitable vehicle was developed making it possible to combine aureomycin and terramycin in a root canal filling cement. Considerable work has been done in vivo and in vitro with this paste. The physical properties of the cement permit its use with gutta-percha and silver points. The cement has the characteristic of flowing under pressure. It is easily introduced, permitting the uniform coating of the walls of the canal. The setting time of between 20 to 30 minutes permits the operator working time to perform an excellent root canal filling. The cement is nonirritating to periapical tissues. In vitro tests over varying periods of time, up to 44 days, show that the cement under different conditions retained its strong antibacterial effectiveness.

47. The X-Ray Diffraction Microcamera and the Specimen Preparation for the Study of Tooth Structure. *Edward Klein, Otto R. Trautz, H. K. Addelston, I. Fankuchen, New York University College of Dentistry, New York, N. Y.* X-ray diffraction methods offer an opportunity to study some phases of the chemistry and crystallography of the enamel of teeth. As the structure varies often within short distances only microbeam methods are considered appropriate. A simplified micro camera is constructed, characterized by a 30 micron slit capillary and 15 mm. specimen-to-film distance. The dimensions for the slit optics are calculated. The orientation of the diffraction pattern in relation to that of the enamel specimen must be known, for which special marks in the camera are provided. A Geiger-Mueller tube and circuit facilitates the alignment of the camera with the x-ray tube. Thin sections of teeth, 150 to 200 microns thick and with the desired orientation, are cut with a diamond saw, then ground and polished by hand to about 40 microns thickness. Or fragments of crushed enamel with only a few rods in the bundle or even individual enamel rods can be picked up by a shellac moistened glass fiber and mounted on a film support. The specimens are examined under the polarizing microscope and a desired location or fragment is centered over the slit capillary. A microphotograph of the specimen is taken for a permanent record of the exact spot where the x-ray penetrates the specimen. The diffractogram obtained with the camera and sample as described above show a high resolution of the diffraction lines with a minimum of background scattering. Even very weak reflections which were not previously demonstrated in a diffractogram of tooth enamel are recorded. (Published in *J. D. Res.* **30**: 439, 1951.)

48. Thin Sectioning of Dental Tissues for Electron Microscopy. *David B. Scott, Marie J. Ussing, and Ralph W. G. Wyckoff, National Institute of Dental Research, National Institute of Arthritis and Metabolic Diseases, and National Institutes of Health, Bethesda, Md.* The dental tissues which thus far have been successfully sectioned for direct electron microscopy include decalcified enamel and dentin, undecalcified enamel and dentin, pulp, periodontal membrane and epithelial attachment. Procedures are described for the decalcification and handling of tissues prior to embedding, and for orientation and single and double embedding in methacrylate. Sectioning is performed through use of a thermal expansion microtome attachment (*J. Res. Nat. Bureau Standards* **43**: 183, 1949), and a glass knife (*Proc. Soc. Exper. Biol. & Med.* **74**: 436, 1950). Electron micrographs show the fine structure of the various tissues sectioned.

VII. Fourth Session, Morning, March 18, Abstracts 49-59

49. Delayed Radiation Injury of the Teeth. *B. O. A. Thomas, Simion T. Cantril, and Robert C. Canfield, University of Washington, Seattle, Wash.* There are definite destructive effects on the calcified dental tissues following their exposure to large dosages of radiation, but no histologic studies of these radiation-damaged teeth have been reported, so far as the authors have been able to determine. The major problem is to determine whether the histologic changes observed are actually dental caries or whether they are lesions peculiar to radiation damage. It has been contended that the radiation injures the salivary glands and that the resulting xerostomia is responsible for the tooth destruction. In the present study, teeth have been recovered from three patients (one woman, two men) treated with heavy dosages of radiation for malignancies of the oral region. In one case, the teeth were removed at postmortem examination; the other two patients are apparently well, three and four years following radiation therapy. Teeth have been prepared for study, using ground sections, decalcified sections (paraffin, celloidin and celloidin-decalcifying methods), and powdered tooth structure for checking tissue radioactivity. This is a preliminary report.

50. The Effect of Certain Chemical Compounds Upon Dentin. *George W. Burnett and John S. Oartel, Dental Division, Army Medical Service Graduate School, Army Medical Center, Washington, D. C.* An investigation has been made of certain chemical compounds which might interfere with the dental carious process by decreasing the solubility of the inorganic ground substance of dentin, or which might decrease the accessibility of its organic matrix. Sections of dentin were exposed to NaF, CaF_2, PbF_2, K_2PO_3F, $CaPO_3F \cdot 2H_2O$, $FeCl_2 \cdot 2H_2O$, $AgNo_3$, HCHO, $ZnCl_2$ (Zin-Ferro No. 2), and $K_3Fe(Cn)_6$ (Zin-Ferro No. 3) for specific periods of time under carefully controlled conditions. After treatment, the sections, together with untreated control sections, were exposed to lactic, acetic, butyric, formic, succinic, citric, hydrochloric, and nitric acid at pH 3.0 for 24 hours at 37.5° C. The accessibility of the inorganic components was measured by loss in weight, while the accessibility of the organic matrix was measured by the diazotization process. The latter process is an indirect measure of the accessibility of the organic matrix for lysis by pepsin or pepsin-like enzymes. The results obtained indicate that $AgNo_3$ and the fluorides, except CaF_2, decrease the solubility of the inorganic matrix. Ferrous chloride has a deleterious effect upon the inorganic substance. Formaldehyde, $ZnCl_2$ and $K_3Fe(Cn)_6$ do not decrease the solubility of the inorganic components but render the organic matrix more accessible for lysis as shown by the method of measurement.

51. Chemical Studies of the Human Enamel Surface. *Finn Brudevold and Marguerite F. Little, Eastman Dental Dispensary, Rochester, N. Y.* Small standard sized areas of intact enamel surfaces were exposed repeatedly to acetate buffer pH 4, and the amounts of dissolved Ca and P were determined using micromethods. Significantly less Ca and P were dissolved in the first than in the subsequent exposures of the same surface. This was true of erupted and unerupted teeth. The Ca/P ratio averaged 1.99, 1.93, and 1.94 in the first, second, and third acid exposure. Since the amount of Ca recovered was about 6 per cent low with the Ca method employed, the true Ca/P ratio must be considered to be correspondingly higher. Repeated acid extracts from intact and ground enamel surfaces frequently showed variations in the Ca/P ratio. This suggests that there may be differences in the inorganic chemistry of the enamel from the same tooth. Fluorides were found to be more effective in reducing

surface solubility when applied to etched enamel surfaces than when applied on intact enamel surfaces. The effect of 2 per cent NaF on the intact surface approached significance (C. R. 1.6), and 15 per cent Na_2FPO_3 and saturated PbF_2 were ineffective. On the etched surface both NaF and PbF_2 produced a marked reduction in solubility, with PbF_2 being significantly more effective.

52. X-ray Diffraction by Human Dental Enamel. *Otto R. Trautz, Edward Klein, H. K. Addelston, I. Fankuchen, New York University College of Dentistry, New York, N. Y.* X-ray diffraction is being employed as a modern research tool in dentistry. Enamel is the best crystallized material in the human body. Interpretation of diffraction patterns is illustrated by diffractograms of enamel. At present attention is concentrated on two problems: (1) the identification of the crystalline constituents in enamel as an aid in the chemical study of the enamel and bone formation; (2) the description of the structural arrangement of the crystalline matter as a supplement to the histological study of both the developing and the mature enamel. Scrutiny of the diffractograms of enamel with all reflections identified, even under the most favorable experimental conditions, indicates only one single apatite and no other crystalline substance in enamel. The apatites are a crystalline form of calcium phosphates having the general composition $Ca_{10}(PO_4)_6X_2$, in which X_2 may be F_2, $(OH)_2$, Cl_2 or O. Some Ca may be replaced by other cations. The placement of the carbonate is still disputed. The ionic replacements are accompanied by changes in the unit cell dimensions. Measurements of the crystallographic axes of the enamel apatite were made. The values are different from those for synthetic hydroxyl apatite. When the enamel is heated (900° C. for 20 hours) a partial transformation always occurs, accompanied by the appearance of new lines, though to a lesser degree than in dentin. The apatite lines exhibit a slight shift towards a lower c/a axis ratio. The structural investigation deals with the arrangement of the crystallites in the fiber structure and of the fiber structure in the enamel. A diffractogram with a double fiber pattern is often obtained.

53. Electron Microscopy of the Organic Framework of the Enamel. *Reidar F. Sognnaes, David B. Scott, Marie J. Ussing, and Ralph W. G. Wyckoff, Harvard Dental School, Boston, Mass., National Institute of Dental Research, National Institute of Arthritis & Metabolic Diseases, National Institutes of Health, Bethesda, Md.* The electron microscopic structure of the organic framework of the enamel has been studied in thin sections of human and animal teeth in various stages of development. A distinctly oriented organic network has been revealed both within and between the prisms of decalcified mature human enamel. Micrographs are presented which demonstrate the character, size, and morphologic orientation of the organic material in the prism core, prism sheath, interprismatic substance, and tufts.

54. Microstructure of the Enamel Cuticles and Epithelial Attachment. *Marie J. Ussing, David B. Scott and Harry Kaplan, National Institute of Dental Research, National Institutes of Health, Bethesda, Md., and Washington, D. C.* Studies have been made of the structure of and the relation between the primary cuticle and reduced enamel epithelium in unerupted teeth, and primary and secondary cuticles and epithelial attachment in recently erupted teeth. Three methods have been employed: (1) optical microscopy of shadowed replicas of cuticle-covered tooth surfaces; (2) optical microscopy of entire cuticles with adherent epithelial cells, isolated and stained by special methods; and (3) electron microscopy of thin cross sections of similar isolated

cuticles. The primary cuticle, secondary cuticle, and overlying epithelium all appear to be interconnected in a fashion similar to the strata of keratinized epithelial tissue, various types of which have also been studied by electron microscopy. It is a possibility that the mechanism of so-called secondary cuticle formation and epithelial attachment may be explained as a process of keratinization, beginning with the ameloblasts, with all of the successive layers being connected together just as is the stratified keratin in other epithelial tissues. This possibility is being further investigated by comparing epithelial tissues in various stages of keratinization with cuticular membranes in various formative stages.

55. Proteinaceous Material in Enamel. I. The Distribution of Proteinaceous Materials in the Enamel of Unerupted, Undecalcified Human Third Molars. *Wm. E. Hutton, and James Nuckolls, University of California College of Dentistry, San Francisco, Calif.* Eight completely unerupted third molars were collected within 8 hours after extraction, washed, and stored at or below 0° C. Distilled water served as a collection, washing, and storing medium. Whole crowns, from which roots and pulps had been removed, were tested directly for distribution of protein by immersion in suitable reagents. A positive biuret test (alkaline copper citrate) indicated protein as such; while ninhydrin (0.25 per cent in n-butanol), since it does not react with keratin, casein, or ammonia in the concentrations found in enamel, was taken as an indicator of short chain polypeptides and amino acids. Various occlusal pits and grooves of 6 of the 7 molars primarily treated with ninhydrin were stained as were those of the one molar treated with biuret reagent. The mesial surfaces of 2 of the 7 ninhydrin-treated molars were stained and buccal pits of 2 of these molars as well. One of the molars was stained by ninhydrin in anomalous lingual pits. Three molars treated with ninhydrin reagent were cut in 2 mm. sections and examined for subsurface stained areas. Two of these molars were stained in the inner two-thirds of the enamel underlying the buccal surface of the crown, and these sections (after discharging the ninhydrin color) also gave positive biuret tests in these same regions. It was estimated that the chances were at most 6 in 100,000 that localization of protein, short chain polypeptides, and amino acids in caries-susceptible surfaces were accidental. The relation between occurrence of these proteinaceous materials in surfaces of teeth and probability of these same surfaces in first molars becoming carious was examined. A positive correlation coefficient of 0.92 was computed for the 39 surfaces included in the study. This coefficient is quite highly significant. The data suggest the possibility that certain proteinaceous components of enamel may contribute to susceptibility of third molars to caries.

II. On the Presence of Nonkeratin Proteinaceous Compounds in Human Enamel. Ninhydrin reagent (0.25 per cent in n-butanol), since it does not react with keratin, casein, or ammonia in the concentrations found in human enamel, may be taken as an indicator of low molecular weight polypeptides and amino acids. When treated with this reagent, seven completely unerupted third molars became stained in various regions of the enamel, indicating the presence of substances less inert than the keratin which is usually believed to be the main organic component of this tissue. Preliminary attempts to confirm this observation has been made by means of another test. Para dimethylaminobenzaldehyde (3 per cent in 5 per cent hydrochloric acid in ethanol 40 per cent, ether 40 per cent) stains amino acids and casein a red purple to a deep blue color while not affecting ammonium sulfate, stains keratin a faint pink or not at all, and does not stain aminopolysaccharides unless they have been subjected to a prior alkaline condensation, for example, with acetyl-

acetone. Thus far 4 tooth crowns have been decalcified with this reagent. Three of these were completely unerupted third molars and one a slightly carious first molar. In these four teeth the patterns of decalcification have been similar. At least some of the occlusal pits and grooves contain a material which stains a red purple to a deep blue color while much of the enamel in the vicinity of the dentino-enamel junction contains material which stains a red purple to a deep blue. The bulk of the enamel matrix, except for occasional pink fibrils, remains unstained. The decalcification may be followed photographically. These results indicate that the enamel of the four teeth examined contains either proteins or breakdown products of proteins which differ from keratin in their greater chemical reactivity.

56. Carbohydrate of Carious Enamel. *Thomas Coolidge, Zoller Dental Clinic, University of Chicago, Chicago, Ill.* Microchemical analysis of sound enamel and of the enamel of early caries of the enamel, before changes in surface contour, show that there is little difference in the carbohydrate content of normal and carious enamel as determined by the carbazole method. This fact seems incompatible with theories postulating that enzymes resembling collagenease or hyaluronidase are involved in caries of the enamel.

57. The Permeability of Human Tooth Enamel. *Carl Wachtl, and L. S. Fosdick, Chemistry Department, Northwestern University Dental School, Chicago, Ill.* There is some information to indicate that the morphological characteristics of the carious lesion in the enamel may be explained if the tooth is considered to be permeable to small molecules. Sound anterior human teeth were selected and the pulp chamber was removed by means of a burr. The tooth was set up in such a manner so as to act as a membrane. The inside surface of the tooth was exposed to distilled water solutions, while the outside was exposed to ammonia, urea, boric acid, and sugar solutions. The permeability of the tooth was studied in relation to osmotic pressure differential between the outside and the inside of the tooth. It was found that small molecules or ions would penetrate the enamel as determined by chemical means.

58. Time Studies of the Penetration of Extracted Human Teeth by Radioactive Nicotinamide, Urea, Thiourea, and Acetamide. II. Penetration of Dentin From the Pulp Chamber. *W. W. Wainwright, and H. H. Belgorod, Radiobiology Section, Los Alamos Scientific Laboratory of the University of California, and the Dental Clinic, Los Alamos Medical Center, Los Alamos, N. M.* Isotope-labeled solutions of nicotinamide, urea, thiourea, and acetamide were tested for their ability to penetrate dentin by placing them in the cleaned pulp chambers of extracted human teeth. Twenty-one unfixed and 5 formol-fixed teeth were treated, stored in a humid atmosphere, and after periods of 6 minutes to 3 days were sectioned under oil and examined radioautographically. C^{14}-nicotinamide, C^{14}-urea, S^{35}-thiourea, and C^{14}-acetamide penetrated dentin rapidly Each compound penetrated through approximately 3.5 mm. of dentin to reach the dento-enamel junction in about 20 minutes. Acetamide penetrated through the dentin (4.1 mm.) and faintly through the enamel (2.1 mm.) within 22 minutes at 37° C.

59. Penetration of I-131 Through Cat Canine Into Systemic Circulation by Continuous Contact of Microdrop of Solution With Enamel Surface. *Herbert J. Bartelstone, Veterans Administration Hospital, Bronx, Columbia University, College of Physicians and Surgeons, New York, N. Y.* Radioiodine was delivered to a very small area of the enamel surface of cat canines at a rate of 0.005 c.c. per hour by means of a continuous flow capillary pipette technique. With this method, fluid containing 1 millicurie per cubic centimeter

of carrier free NaI-131 in H_2O is added to the microdrop at a rate which is equal to, and determined by, the loss of fluid from the drop due to evaporation and absorption. Radioautographs demonstrate the penetration of I-131 through the enamel with uptake of I-131 by the tooth, periodontium and thyroid gland which is used as an indicator for systemic uptake. Controls are described which rule out possible distribution artifacts due to the sectioning procedures.

VIII. Fifth Session: Section A. Afternoon, March 18. Abstracts 60-73

60. End Point Study of Bactericidal Effect of Antibiotics. *Louis I. Grossman and K. Christian, School of Dentistry, University of Pennsylvania, Philadelphia, Pa.* The use of a polyantibiotic mixture such as penicillin, streptomycin, bacitracin, caprylate sodium, in root canal therapy made the determination of the bactericidal end points of each component desirable. Graded concentrations of the antibiotics were tested against serial dilutions of the various test organisms. Bacteria used were representative of the most frequent strains isolated from infected root canals.

61. Experimental Studies of Focal Infection in Monkeys: The Systemic Effects of Hemolytic Streptococci in Root Canals of Teeth. *Donald R. Kennedy, Tom R. Hamilton, James Jensen, and Jerome T. Syverton, Department of Oral Surgery, School of Dentistry, and Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.* The relationship in dental surgery of focal infection to bacteremia, rheumatic disease and specific alterations in streptococcal antibodies is little understood. Accordingly, this experimental study was designed to learn whether introduction of streptococci into the root canals of teeth in monkeys would lead to measurable changes. The first experimental series included 11 monkeys, 6 of which were inoculated alternately with beta-hemolytic streptococci, Groups A and C, smooth phase, and 5 of which served as uninoculated control animals. Pulp was removed from the root canals of the anterior teeth of all monkeys. Teeth in the test group were inoculated with the cultural sediment suspended in FALBA. No bacteremias were established following any of these procedures. Blood counts and erythrocyte sedimentation rates gave no evidence of systemic alterations. A second experimental series employing 3 monkeys was altered in plan by using Group A streptococci, matt phase, as different serologic types. Bacteremia resulted regularly. Observations on these animals demonstrated in one monkey: (1) systemic effects, as manifested by fever, leucocytosis, and an elevated erythrocyte sedimentation rate; (2) a serologic response, as measured by titration of antistreptolysin O; and (3) a histopathological response in evidence of cardiovascular disease, "rheumatic type." The experimental approach for a third series was modified by employing for inoculation hemolytic streptococci in the mucoid phase. The results of our studies in these animals will be reported.

62. Effects of the Oral Bacterial Flora on Tetanus Toxin Formation. *Holmes T. Knighton and Edward H. Martin, School of Dentistry, Washington University, St. Louis, Mo.* Approximately 2000 tetanus spores were inoculated in each of numerous, freshly autoclaved, tubes of Difco's heart infusion broth containing 0.1 per cent agar. Each tube (⅝ inch diameter) contained 10 c.c. of medium. *Cl. tetani* was grown in pure culture in one set of tubes, others were inoculated with 0.1 c.c. amounts of either (a) untreated paraffin stimulated saliva or (b) broth cultures of *Staphylococcus aureus, Streptococcus viridans* or *Leptotrichia buccalis.* Cultures were incubated at 37° C. for 7 days, passed through Seitz filters, and varying amounts of filtrates injected intramuscularly

into hind legs of mice. Antitoxin-protected mice were used as controls in each series. The smallest amount of toxin killing between 96 and 120 hours was considered as the minimum lethal dose (M.L.D.). Results were as follows: (a) pure cultures of *Cl. tetani* produced approximately 30,000 M.L.D. per c.c., (b) toxin formation was not significantly reduced by growth of either *Staph. aureus, Str. viridans* or *L. buccalis,* (c) mixed culture of bacteria contained in paraffin stimulated saliva either inhibited *Cl. tetani* growth and/or reduced toxin formation from 67 to 100 per cent. Further experiments are necessary to determine the specific organisms responsible for the antagonistic effect.

63. The Occurrence of Micrococcus lactilyticus in the Dento-Bacterial Plaque. *J. J. Ennever, School of Dentistry, University of Kansas City, Kansas City, Mo. Micrococcus lactilyticus,* a lactate utilizing anaerobe, has been shown to be a member of the normal oral flora. Preliminary to investigating a possible relationship between cariogenesis and this microorganism, it was deemed essential to determine the occurrence in the dento-bacterial plaque. Plaques obtained from 18 clinically normal subjects, cultivated on yeast extract-lactate agar, anaerobically at 35° C. gave consistent isolations of *M. lactilyticus.* Examination of smears following Hucker's modification of the Gram stain revealed the microorganism to be gram-positive in very young culture. Thus, inclusion of *M. lactilyticus* within the genus Veillonella, as has been suggested by previous authors was indicated to be invalid. (Published in *J. D. Res.* **30**: 423, 1951.)

64. Effect of Lysozyme on Micrococcus Lysodeikticus. *J. L. Nemes and M. G. Wheatcroft, Naval Medical Research Institute, Bethesda, Md.* The effect of lysozyme on *Micrococcus lysodeikticus* has been controversial when studied by light microscopy. Recent evidence under phase and electron microscopy has shown these susceptible cells to be doublets, to possess internal organization, internal structures and a degree of lysis which varies according to method of observation.

65. Caries In Vitro: I. Production of Microscopic Carieslike Lesions With Broth Cultures of Bacteria Associated With Caries. *Valerie Hurst, James Nuckolls, Harry E. Frisbie and Max S. Marshall, University of California College of Dentistry, San Francisco, Calif.* Tests are being continued on the ability of bacteria commonly associated with caries to produce microscopic carieslike lesions in unerupted hamster molars at neutral pH. As of December, 1950, 13 strains of actinomycetes isolated from plaques had produced lesions in 36 out of 80 teeth. Eleven strains of lactobacilli, obtained from several laboratories, had produced lesions in 30 of 72 teeth. Four strains of oral alpha streptococci had produced lesions in 7 out of 20 teeth. All lesions occurred at neutral pH values. In an additional 32 tests 2 per cent dextrose was added to broth cultures of lactobacilli and alpha streptococci. The enamel was usually almost completely dissolved at the acid pH values produced, but in 10 of these molars evidence was found in the enamel remnants that penetration of the enamel may have occurred. These results suggest that at least several groups of bacteria possess the ability to utilize organic substances of the enamel matrix in their nutrition, and that the initiation of enamel caries by any one of a number of oral bacteria may be possible. Also, these results would indicate that the initiation of caries may occur independently of acid decalcification and the presence of fermentable carbohydrate in the environment.

II. Production of Microscopic Carieslike Lesions With Washed Cell Suspensions of Bacteria Associated With Caries. To determine whether the microscopic carieslike lesions described in Part I were the result of utilization of

the enamel matrix exclusively, similar tests have been performed with the bacterial cells washed and suspended in physiological salt solution. Difficulties encountered have been the limited survival and abnormal staining properties of the bacteria subjected to this treatment. Nevertheless, definite carieslike lesions were produced by one actinomycete strain in 3 of 29 molars. Probable lesions occurred in 5 other molars of this series. One lactobacillus strain produced probable lesions in one out of 10 molars on which lactobacilli were tested. In a further experiment the bacterial cells were suspended in Seitz-filtered or autoclaved saliva after washing. Definite lesions were again obtained in each of 2 molars with 2 actinomycete strains. Suggested, but less definite lesions, were obtained in one molar with a lactobacillus. These results indicate that certain bacteria may utilize the organic enamel matrix as their sole nutrient source.

III. Failure to Produce Microscopic Carieslike Lesions With Broth Cultures of Bacteria Infrequently Associated with Caries. Various bacteria not frequently related to caries etiology have been tested for their ability to produce microscopic carieslike lesions in vitro. The tests performed thus far have included *Staphylococcus albus, Staphylococcus aureus, Sarcina lutea, Bacillus subtilis, Bacterium coli, Zopfius zenkeri,* and unclassified diphtheroids obtained from throat swabs. No evidence of lesions has been found in the 33 molars tested with these bacteria. These tests included 6 molars for which the corresponding molars of litter mates were attacked in vitro by an actinomycete under similar conditions. In molars of 2 of the animals no lesions were obtained with *S. lutea* or *Staph. albus* while lesions did develop in the other molars of these same animals tested with an actinomycete. Such results suggest that the ability to attack the enamel matrix may characterize the bacteria which have been most commonly reported to be associated with the carious lesion.

66. In Vitro Dental Decalcification Resembling Caries: A Preliminary Study. *F. C. Besic, Walter G. Zoller Memorial Dental Clinic, University of Chicago, Chicago, Ill.* The prolonged effect of bacterial metabolism upon portions of the crowns of 3 teeth from 3 different individuals was observed. Tooth A was extracted from a patient with rampant caries, tooth B from an immune mouth and tooth C, showing mottled enamel, is of unknown origin. The teeth were covered with dental sticky wax except for areas on the proximal and buccal surfaces, were mounted in modeling compound, and one c.c. of fresh saliva was used to contaminate a mixture of Brewer's media and bacto-dextrose into which was inserted the block containing the teeth. The contaminated mixture was incubated and renewed weekly. After 24 hours of incubation pH readings averaged 4.5. The experiment was continued for 6 months. Radiographically typical carieslike penetration was seen throughout the enamel in teeth A and B. Tooth C (mottled enamel) showed shallow and atypical rarefaction. Surface continuity, as well as surface gloss, was preserved except in the buccal areas which were pierced with a dental explorer to denote softening of the tooth substance. The color of the affected areas was grayish brown. Excavation of the buccal region of tooth C showed the dentin to be soft, leathery and discolored dark brown. The softened dentin spread at the D. E. J. undermining the enamel. This study neither proves nor disproves that bacteria are responsible, either in part or in whole, for dental caries. It does suggest that bacterial metabolism studies might be employed to determine certain aspects of carieslike disintegration of tooth structures in vitro. The successful preservation of surface continuity in this study suggests that factors in addition to pH are necessary for the process since pure acid solutions per se, at any pH, destroy surface contour.

67. Further Studies on In Vivo Decalcifying Agents. Versene (ethylene diamine tetra acetic acid), Various Polycarboxylic Acids and Various Polyphosphates at pH 6.5. *I. Zipkin, National Institutes of Health, Bethesda, Md.* Since it has been shown by McClure and Ruzicka that citrates at pH 6.5 will decalcify rats' molar teeth in vivo, other compounds possessing possible calcium binding (sequestering) properties have been investigated. The calcium complexes so formed must be soluble and non-ionized at a neutral pH. Various polyphosphates, hydroxypolycarboxylic acids and aminopolycarboxylic acids appear to fulfill these requirements. All decalcification studies were carried out for 120 days at pH 6.5 with only few exceptions. The rats' molar teeth were scored according to the method of Restarski, et al. The solutions were administered to the rats as the sole source of drinking fluid. Versene, which proved to be the most potent decalcifying agent tried to date, was further studied with both in vitro and in vivo experiments. It was shown that as much as 100 ppm F failed to inhibit the decalcification produced by the 0.25 per cent Versene solution both in vitro on powdered rat enamel and in vivo on rats' molar teeth. Analyses of the rat enamel from both experiments are in progress in an attempt to elucidate the mechanism of the decalcification. Preliminary experiments have also shown that Versene solutions may be superior to acids as decalcifying agents in the preparation of intact dentin and enamel for sectioning and histological examination.

68. The Development of Caries on The Buccal and Lingual Tooth Surfaces of Rats As Well As Proximal and Fissure Caries. *Robert M. Stephan, National Institute of Dental Research, National Institutes of Health, Bethesda, Md.* Hoppert described the importance of the particle size of diets in the production of occlusal surface fissure caries in rats, and Rosebury described, in addition, a form of proximal surface caries. However almost no references have been made to the occurrence of caries on the buccal and lingual surfaces of the teeth of rats, although Arnold has described such carious lesions in hamsters. In the present study, a rampant form of smooth surface caries, similar in location to gingival third caries in humans, has been observed on the buccal and lingual surfaces of both the upper and lower molar teeth of albino rats, in addition to proximal surface and occlusal fissure caries. These buccal and lingual surface carious lesions begin as a white etched area on the enamel surface, and frequently extend through the enamel and into the dentin, with a typical secondary spread of caries at the dentino-enamel junction, and extensive cavity formation. Of 249 weanling Sprague-Dawley strain rats placed on low fat, high sugar diets consisting chiefly of skimmed milk and cane sugar supplemented with cod liver oil, 389 carious lesions were produced on the buccal or lingual surfaces in 74 of the animals, 281 proximal surface lesions were produced in 46 of the animals, 449 occlusal fissure lesions were produced in 98 of the animals. Seventy-one animals were caries free, after 100 days, and 38 animals died before a caries score could be made. In other experiments with this strain of rats on different diets, no buccal or lingual surface caries have been observed in 254 animals on a synthetic, high sugar diet, or in 208 animals on a modified Hoppert-Webber-Canniff coarse particle diet. In an attempt to define the factors which are associated with this unusual type of rat caries, it was found that the greatest amount of buccal and lingual surface caries occurred in individual animals which failed noticeably to gain weight. On the other hand occurrence of occlusal fissure caries showed no such correlation.

69. Some Observations Concerning Dental Caries Production in the Syrian Hamster. *J. F. Volker, University of Alabama, School of Dentistry, Birmingham, Ala.* The influence of age on the production of experimental caries

in the Syrian hamster has been studied in over 400 animals. The following observations have been made: (1) Hamsters maintained on a noncariogenic diet from birth until the forty-fifth day have been placed on a modified H. W. C. diet (sucrose completely replacing coarse corn) for 100 days. Upon sacrifice, a limited amount of caries has been observed. The most severe lesions occur in the third molars. (2) Hamsters maintained on a noncariogenic diet until the twenty-fifth to the thirtieth day have been placed on a modified H. W. C. diet (sucrose completely replacing coarse corn) and kept for 100 days. Moderate caries results. Although the greatest amount of tooth decay occurs in the third molars, the first and second molars are also usually involved. (3) Nursing hamsters 8 to 12 days of age have been permitted access to a modified H. W. C. diet (sucrose completely replacing coarse corn). At approximately the twenty-fourth day, they have been weaned and continued on the aforementioned ration. Severe caries develops within 45 days. In the average instance, extensive caries develops in all of the molar teeth. It should be noted that the regime used in (3) is comparable to the situation in human beings where children have access to refined carbohydrates before and during the eruption of the molar teeth.

70. Geographic Variation of the Cariogenic Effect of Similar Foodstuffs: I. Riboflavin's Role. *Abraham E. Nizel, Tufts Dental School, Boston, Mass., and Robert S. Harris, Mass. Institute of Technology, Cambridge, Mass.* Previously (*Arch. Biochem.* **26**: 155, 1950), we have reported a statistically significant (40 per cent) difference in caries in hamsters fed corn and milk produced in northwest Texas in comparison with corn and milk produced in New England. These findings have been confirmed (*New England J. Med.*, March, 1951), and it has been demonstrated, furthermore, that the New England corn and milk both exerted cariogenic effects that overwhelmed any inhibitory action of Texas foodstuffs. It appears that there is some mechanism or substance common to New England corn and milk which materially aided in the production of dental decay. In a third feeding experimental series the same significant decrease of caries was found in the group fed the Texas diets in comparison with the New England diets. The corns and milks from both areas were analyzed for 13 essential nutrients. The only important difference was in the content of riboflavin, of which the New England foods were 30 per cent richer. To determine whether this difference in riboflavin (0.2 mg. per 100 Gm. diet) affected the cariogenicity of these diets, 2 groups of 30 hamsters each received Texas diets enriched with 0.2 mg. riboflavin and 2.0 mg. riboflavin per 100 Gm. diet. A third group received the New England diet. The results showed that addition of 0.2 mg. riboflavin caused a slight but insignificant decrease in the cariogenicity of the Texas diet, and that the addition of 2.0 mg. riboflavin caused a very significant reduction in caries prevalence. It is evident that the higher cariogenicity of the New England diet was not due to its higher riboflavin content. A study of the role of riboflavin in dental caries is under way.

71. Caries Production in Hamsters by Different Foods. *Basil G. Bibby, Hyman J. V. Goldberg and Eugene Chen, Eastman Dental Dispensary, Rochester, N. Y.* In an attempt to correlate the "decalcification potential" of various foods with caries production in animals, figs (high potential) and carrots (low potential) were fed 6 days per week to 19 hamsters for 90 days. Results with figs and carrots were respectively: average caries score, 9 and 74; average number of cavities, 10 and 8. In a second study three foods, (a) sprayed cracker, (b) graham cracker and (c) white bread, were incorporteed as 60 per cent of a diet containing wheat germ (10 per cent), powdered milk (20 per cent), cellulose (5 per cent), alfalfa (4 per cent) and sodium chloride (1 per

cent). Fifty hamsters were fed the above diet in unlimited quantities with distilled water for 86 days. With the three foods the average caries scores were, respectively, (a) 19, (b) 32, (c) 7; and the number of cavities (a) 12, (b) 16, (c) 11. Direct parallelism does not appear to exist between "decalcification potentials" and caries production in hamsters.

72. Influence of Salivary Gland Extirpation on Development of Dental Caries in the Syrian Hamster. *C. E. Klapper, University of Alabama, School of Dentistry, Birmingham, Ala.* In these laboratories it has been observed that hamsters maintained on a noncariogenic laboratory diet until 45 days of age resist the development of dental caries when placed on a modified H. W. C. diet (sucrose completely replacing coarse corn). When caries does occur, it is confined primarily to the third molars. In this study, 30 adult Syrian hamsters, 45 to 70 days of age, had the major salivary glands removed. Prior to the operation they were fed a noncariogenic laboratory diet. For a period of approximately 100 days after the operation they were maintained on the modified H. W. C. diet (sucrose completely replacing coarse corn). On this regime, severe caries developed in almost all of the molar teeth. The greatest destruction has been observed in the first molars and the least in the third molars. The severity and pattern of the experimental caries is in sharp contrast to that observed in nondesalivated animals and points to the important protective role of saliva in the production of dental caries in the adult Syrian hamster.

73. Ammoniated Dentrifices and Hamster Caries: II. The Effectiveness of Brushing the Teeth With an Ammoniated and Control Dentifrice. D. S. *Chernausek and David F. Mitchell, University of Minnesota, School of Dentistry, Minneapolis, Minn.* This study was collateral with one previously reported (The Effect of Ingestion). In essence, three groups of animals were employed: G-I control (14 ♂, 14 ♀); G-II control brushing, neutral dentifrice (14 ♂, 13 ♀); G-III experimental brushing, ammoniated dentifrice—5 per cent dibasic ammonium phosphate and 3 per cent urea—(11 ♂, 12 ♀). The animals were maintained for 100 days on a high carbohydrate diet. The teeth of the animals in groups II and III were brushed daily. The average total caries scores (♂ + ♀) were: G-I 41.9; G-II 20.1; and G-III 14.8. The male scores were consistently higher than the female scores. Statistical evaluation of the data indicates a significant reduction of caries by toothbrushing per se.

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74. Pulp Recovery From Heat Injury. *Vincent F. Lisanti and Helmut A. Zander, Tufts College Dental School, Boston, Mass.* The immediate response of the pulp to heat injury has been described previously. The purpose of this study was to determine whether or not the pulp could recover from the initial injury. Cavities were prepared in 100 teeth of 11 dogs. Temperatures ranging from 125° to 600° F. were introduced on the dentin surfaces to produce injury. The teeth were obtained for sectioning at various time intervals up to 2 months after heat injury. The cavities were filled for this period with zinc oxide and eugenol. The pulp was able to recover in all instances with secondary irregular dentin deposition.

75. Lymphocytes In the Epithelium of The Healthy Gingiva. *Martin Cattoni, Northwestern University Dental School, Chicago, Ill.* Lymphocytes were found to be constantly present in the epithelium of the healthy gingiva of dog and man. In biopsy material these cells were found passing through the basal membrane, entering into the intercellular spaces and pushing aside the

cells of the basal epithelial layer. In this position both lymphocytes and basal epithelial cells showed a physical distortion. When the lymphocytes pass through the basal layer their normal shape reappears. Few lymphocytes were found but their presence was constant in all of the specimens studied. Most of the lymphocytes were present in the germinative layer and fewer in the prickle cell layer. The lymphocytes never reach the superficial part of the epithelium. The following changes were observed in the cytoplasm and nucleus of the lymphocytes. The cytoplasm became both wider and clearer while the nucleus stained more deeply, but in some cases this migration was accomplished with slight changes in cell area and morphology. No mitotic figures were observed among the lymphocytes. All the lymphocytes seen were intercellular in position. The connective tissue underlying the epithelium showed no definite signs of injury. There were no indications of lymphocyte or plasma cell proliferation. (To be published in *J. D. Res.*)

76. A Report of Experiments Concerning Bone Growth and Repair. *Warren R. Schram and Enos Jones, Department of Oral Surgery, Northwestern University Dental School, Chicago, Ill.*—Chemically pure salts in the proportion in which they are found in bone were incorporated into a solution of U. S. P. gelatin in water. This product was autoclaved and used as a paste. It was also desiccated and used as a rigid material. The effectiveness of the paste was studied by placing it in a series of bone defects created surgically in the ulna of several dogs. In the clinical use of this material it has been found useful for two purposes: To fill the space left in bone following the enucleation of dental cysts; and for the elimination of excessive undercuts in the bone of the lower arch of the edentulous jaws. The rigid material has been used in animal experiments only. This material has been fashioned into tubes of suitable size and placed in surgically created defects. Two centimeters of the radius with its periosteum has been removed from a large series of dogs. In the treated cases, the tube was placed in the breach as an artificial bone graft. The healing process has been followed by periodic radiographs and, as the dogs were sacrificed, the material within the area of repair was prepared and studied microscopically. We were successful in obtaining a union between the two cut ends of bone in the treated cases in a fair number of experiments. None of the untreated cases showed a union.

77. The Effects of Vitamin D and the Parathyroids on Blood Calcium and Phosphorus in Rats Subjected to Mineral Stress. *Roy O. Greep and Clary J. Fischer, Harvard School of Dental Medicine, Boston, Mass.* Synthetic diets of widely different calcium and phosphorus composition were fed with and without vitamin D to intact and parathyroidectomized rats over a period of 4 weeks beginning at 28 days of age. The operations were performed on the first days of the experiments. Vitamin D tended generally to raise both blood calcium and phosphorus. In intact rats the effect of vitamin D on calcium level became increasingly greater as the calcium intake diminished and disappeared with excess calcium intake, and a similar relationship was observed with phosphorus, except that with excessive phosphorus intake the blood phosphorus was sometimes higher with vitamin D absent. In parathyroidectomized rats, vitamin D produced fairly uniform blood calcium and phosphorus increments without regard to level of intake, suggesting some interesting inter-relationships (integrative and protective) between vitamin D and parathyroid function. The intact rat given vitamin D is able to maintain a normal, or near normal, blood calcium in the face of dietary calcium restriction except in extreme degree. This homeostatic mechanism is impaired by the absence of vitamin D. The blood content of phosphate is subject to greater deviations through alterations of intake than is

calcium. In parathyroidectomized rats the blood calcium and phosphorus content reflects significantly the dietary intake of these elements. Changes in blood phosphorus have a marked tendency to alter blood calcium levels in the opposite direction. On the contrary, changes in blood calcium, however, produced, do not appear to exert a reciprocal and opposing influence on blood phosphate level.

78. Observations on the Effects of Daily Administrations of Cortisone in Young White Rats. *Pierre Leroy and L. V. Domm, Department of Anatomy and the Walter G. Zoller Memorial Dental Clinic, University of Chicago, Chicago, Ill.* Cortisone (Cortone, Merck) was administered in various ways for periods of 7 to 60 days beginning between 1 and 16 days of age. The quantity administered varied from 0.25 to 2.50 mg. Day old rats receiving 0.25 mg. tolerated only 14 daily injections. They revealed reduction in size and weight, skeletal modifications, roughened fur, and sluggishness. The thymus and adrenals were reduced. Experimentals averaged 7.5, controls 26.8 grams. Day old rats receiving 0.75 mg. died after 7 injections—averaging 5.54, controls 12.62 grams. Nine- and 16-day-old animals received 2.50 mg. daily. The former did not survive after 10 injections, whereas the latter received 24. All lost weight. The youngest experimentals averaged 10.0, controls 29.65 grams; oldest 22.5, controls 80.0 grams. The adrenals and spleen were smaller and the kidneys abscessed. Five-day-old rats were fed 1.25 mg. for 27 days. Experimentals averaged 45.23, controls 67.38 grams. The fur was rough, adrenals were somewhat reduced, and the thymus and spleen appeared normal. In 11-day-old animals 2.50 mg. was daily massaged into the skin of the sacral area. The amount administered was gradually increased to 6.0 mg. and the fur became rough. After 36 days the treated area was plucked in some, shaved in others, without varying treatment—26 days later no difference was detected in the hair growth of experimentals. The former averaged 126.25, the latter, 186.0 grams. The lower incisors were massaged with 2.50 mg. daily for 28 days beginning at 9 days. There was a roughening of fur and loss of body weight—averaging 56.45, controls 78.0 grams. The effect on incisors, whose growth was modified, is being further investigated.

79. Effect of Cortisone on Rat Fetuses. *L. V. Domm and Pierre Leroy, Department of Anatomy, and Walter G. Zoller Memorial Dental Clinic, The University of Chicago, Chicago, Ill.* In a previous communication (*Anat. Rec.*, 109: 59, 1951), we reported results of experiments where cortisone was injected directly into fetuses. We concluded that development and parturition were apparently not affected, and we presumed that placental circulation was responsible for absence of specific growth effect on fetus. To verify this conclusion we undertook a study of the effect of cortisone (Cortone, Merck) on fetuses when pregnant mothers instead of fetuses are injected. One pregnant rat received 6.25 mg. of cortisone daily subcutaneously from 18th day of gestation, when laparotomy revealed twelve fetuses, to parturition. On 21st day, five living and two dead young were born. Average weight was 5.62 grams. Three pregnant rats were injected daily subcutaneously with 5 mg. of cortisone, beginning on the tenth, sixteenth and eighteenth days of gestation, respectively. The 2 more advanced mothers were sacrificed 4 days later. Uterine horns were empty but revealed signs of recent placentation. The third rat in which injections were begun on 10th day, had not delivered after 13 days of treatment, so fetuses were recovered by cesarean. All fetuses were living but small and x-rays of the skulls revealed a lower degree of calcification than normal. Average weight was 2.88 grams for experimentals and 5.8 for controls. In another

experiment, 3 pregnant rats daily received 3 mg. of cortisone subcutaneously beginning on thirteenth, fourteenth and seventeenth days of gestation respectively. Normal litters were delivered by each female at term. All young survived even where injections to the mother had been continued during suckling period. We conclude that high dosages of cortisone in pregnant rats have a noticeable effect on fetuses, causing mortality and loss of weight, whereas lower dosages may have no effect. Studies of effects on developing teeth and skeleton are in progress.

80. A New Histochemical Reaction for Calcium. *Valerie Hurst, William E. Hutton and James Nuckolls, University of California College of Dentistry, San Francisco, Calif.* The von Kossa technique is usually employed to demonstrate tissue calcification. In this technique the opacity of the deposited silver results in some loss of structural detail. The reaction is nonspecific and phosphates, carbonates, halides, fatty acids, and proteins may be stained. This causes some discoloration and loss of cytological detail in the uncalcified tissues. An alternative method has been developed which overcomes some of these disadvantages. The stain depends on the precipitation of calcium ammonium ferrocyanide ($Ca(NH_4)_2Fe(CN)_6$) with potassium ferrocyanide ($K_4Fe(CN)_6$) in ammonium acetate solution. This precipitate, when treated with $FeCl_3$, forms Prussian blue. Magnesium gives a reaction similar to calcium. The undecalcified sections are placed in an ammonium acetate (10 per cent w/v) buffer (pH 5, 40 per cent ethanol) containing about 1 per cent $K_4Fe(CN)_6$ for 30 seconds. They are then washed with several changes of the ammonium acetate buffer (30 per cent ethanol) to remove the excess $K_4Fe(CN)_6$. On flooding with 10 per cent $FeCl_3$ for about 5 minutes a deep blue color develops in the calcified areas while uncalcified areas either do not stain or become only faintly blue. The blue color of even the most densely calcified areas is semitransparent. Calcified areas stained blue by this method correspond with those in adjacent serial sections stained by the von Kossa technique.

81. Studies on the Carotid Sinus With Reference to the Syndrome of Ludwig's Angina. *H. Henry Weisengreen, and Henry W. Elliott, University of California, College of Dentistry and Pharmacology, San Francisco, Calif.* References are found to the role of the carotid sinus in the production of dyspnea and terminal respiratory failure encountered in patients suffering from Ludwig's angina. The view has been expressed that in the presence of a Ludwig's induration, the carotid sinus is hypersensitive and that pressure exerted on this organ may induce a fatal apnea. Weese produced suppurative indurations in dogs by injection of turpentine over one of the carotid sinuses and found that pressure applied over the affected sinus could produce apnea under conditions of light barbiturate anesthesia, but apnea could not be induced by pressure applied to the opposite, unaffected carotid sinus. Pressure over either sinus produced no apnea when the anesthesia was deepened, which is supposedly due to paralysis of the carotid sinus mechanism by the anesthetic. In the human being it appeared extremely doubtful that the respiratory disturbances seen in this entity could be ascribed to carotid sinus effects so the condition was reinvestigated in dogs using both the surgically isolated carotid sinus and Weese's preparation. Two groups of dogs were used. In 3 animals the effects on the respiration due to varying the pressure on the surgically isolated carotid sinus were studied. Anesthesia was induced by intraperitoneal injection of 25 mg. per kilogram of sodium pentobarbital and a femoral artery was cannulated for recording blood pressure. The opposite femoral vein was isolated for intravenous injections as required. An endotracheal tube was inserted and connected to a

tambour for recording respiratory rate. The common carotid arteries were located by blunt dissection and followed to their bifurcation. Loose ligatures were placed around internal and external carotids just above the carotid sinuses and around the common carotids below the sinuses. Thus, by applying tension to proper ligatures, pressure in carotid sinuses could be increased or decreased at will. The anesthesia was allowed to lighten and was maintained at a level just below the point at which the animal could make spontaneous limb movements. The second group of 4 dogs was prepared in a manner similar to that of Weese. The respiratory rate was determined by counting the respiratory fluctuations in the blood pressure. The effects of manual pressure on the carotid sinuses, affected or not by turpentine indurations, were observed at levels of anesthesia varying from a point at which there was shivering and response to pain to deep surgical anesthesia. Results were obtained by increasing or decreasing the pressure in the surgically isolated carotid sinuses. These results were repeated many times on each of 3 animals at varying levels of anesthesia, and prolonged apnea was never observed. Instead, an increase in respiratory rate was the more common finding. Neither apnea nor paralysis of the carotid sinuses were ever demonstrated. Our experiments do not substantiate the view that pressure on a carotid sinus involved in an induration similar in clinical appearance to Ludwig's angina could produce an apnea of sufficient duration to be fatal either in the animal or the human patient.

82. Experimental Studies on the Development of the Mandible in the Amblystoma Punctatum. *Barnet M. Levy and Samuel Detwiler, School of Dental and Oral Surgery, Columbia University, New York, N. Y.* A series of experiments were performed to determine whether the axis of mandibular growth is determined at an early stage and whether the regional location of this arch has any influence on tooth formation. The problem of healing and regeneration of the mandible was also studied. A series of operations were done on embryos of *Amblystoma punctatum*, in the late tail bud stage. In one group the mandibular arch of one side was rotated through 180 degrees on the transverse axis. In another group this arch was transplanted to the opposite mandibular arch area of a host embryo, thus rotating only the A-P axis. In a third group, the left or right arch from a donor embryo was transplanted to the anterior somite region of a host embryo. A series of incisions was also made along one boundary or along a combination of two or more boundaries of the mandibular arch. The results of these studies reveal the following: (1) The mandibular arch is incapable of regeneration. (2) Wound healing is slow and frequently incomplete. (3) The A-P axis is already determined at the tail bud stage. (4) Teeth will develop in heterotopically grafted mandibles. (5) Both the teeth and Meckel's cartilage are apparently neural crest derivatives. (To be published in *J. D. Res.*)

83. Embryology of the Teeth. *James K. Avery, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* By means of serial sections of developing pig and human teeth several new aspects of the process of dentinogenesis and amelogenesis have been observed. In the bell stage of development of the tooth the inner enamel epithelium begins invaginating the enamel organ in the region of the future cusps. Further invagination has been noted with the beginning of dentin deposition in the cusps. This developmental process displaces the stellate reticulum so that as the beginning stages of amelogenesis occur in the cusps, the stratum intermedium is in contact with the outer enamel epithelium, not separated from it by a band of stellate reticulum, as has been frequently described. It was also found that as a result of the above process

the ameloblasts and stratum intermedium are in intimate contact with the capillaries of the crypt in the region of the cusps as amelogenesis begins. Further, enamel matrix production does not begin until this contact has become established. The role of the stellate reticulum is apparently mainly that of a space maintainer for the developing crown.

84. The Phylogenetic Evolution of the Teeth From Shark to Man. *T. L. Patterson, Departments of Physiology, University of Detroit School of Dentistry, Wayne University College of Medicine, Detroit, Mich., and Hopkins Marine Station, Stanford University, Pacific Grove, Calif.* Previous observations made during gastrointestinal studies on the shark (dogfish—*Squalus californicus*) focused attention on the relationship of tooth development from an integumental scale. Further study indicated that the rather irregularly placed teeth including those in reserve were arranged in several diminishing rows, becoming continuous over the edges of the jaws with the scales of the integument. When the teeth become fully developed and used up, they fall out, like hairs, and are replaced by new ones, a condition normally existing among the lower vertebrates in which there is a succession of teeth throughout life. The teeth of the shark are embedded in a cartilaginous jaw. They are triangular in shape, very sharp, and show a very decided slant in the caudardward direction along with the body scales. Histological studies made of these scales showed that they are of the placoid type consisting of a flat, basal plate of dermal origin embedded in the skin and bearing a very minute projecting point or spine tipped with enamel and resembling the structure of teeth. The primordial fishes from which modern vertebrates are descended were covered by primitive projecting scales like their modern relatives the sharks. It is assumed, that, as animal life developed, a division of labor appeared among the descendants of the ancestral fishes in which the placoid scales lost the projecting spine but retained the basal plate and these became the scales of modern fishes, snakes, and lizards, which may be seen in a vestigial form in terrestrial animals like the scales on the mouse's tail. The scales located on the jaws, however, were used to hold and tear the animal's prey, so that the projecting spines developed enormously in comparation with the basal plate and in the course of time migrated into the mouth and became teeth. The human tooth is a transformed fish scale and whoever describes a shark's scale is describing a human tooth and vice versa.

85. Growth of the Upper Face Following Bilateral Mandibular Condylar Resection. *Joseph R. Jarabak and T. R. Graber, Northwestern University Dental School, Chicago, Ill.* A study of the growth of the upper face of the rat following bilateral resection of the mandibular condyles was made. For the purposes of linear and angular appraisal, cephalometric radiographs were taken of the occlusal and lateral aspects of the skull. The resection was performed on 30-day-old rats. They were subsequently x-rayed 6 weeks, 12 weeks, 18 weeks, 24 weeks, 26 weeks and 30 weeks following resection. The cranium was divided into two parts at the presphenoidal suture—the anterior portion comprising the face and the posterior, the brain case. There was an increase noted in the height of the face of the operated rats from the occlusal surface of the upper molar to the apex of the ethmoid bone. This increase may be attributed to the change of the occlusal plane following resection. The angle of the occlusal plane increased 4½ degrees. (Fourteen degrees control—18.5 degrees experimental.) No change was noted in the angular measurements of the snout following the resection.

86. The Regeneration of the Mandibular Condyle Following Bilateral Resection. *Joseph R. Jarabak, Northwestern University Dental School, Chi-*

cago, Ill. A histological study was made on the structure of the regenerated area of the mandibular condyles of the rat. The condyles were resected bilaterly on 30-day-old rats. Specimens were taken 12 days, 36 days, 50 days, and 78 days after resection. In the first group, the area on the neck immediately below the resection showed a number of empty lacunae. There was extensive osteoblastic activity noted below the above mentioned area. The region above the section was composed of an atypical mass with a few cartilage cells and an atypical fibrous structure extending to the remnants of the remains of the articular capsule. In the specimen taken 36 days after resection, the callus began to form definitive areas of cartilage extending from the neck of condyle to the fossa. In the 50-day specimen the cartilage cells appeared along the superior aspect of the modified or reformed condylar surface with a new formation of cancellous bone below. In the 78-day specimen, the cartilage layer was very thin with extensive formation of cancellous bone. There were also irregular areas in the cartilage which were being invaded by osteoblasts and capillaries from the cancellous bone. The regenerative process of the mandibular condyle seems to simulate the repair of a fracture of an endochondral bone.

87. Growth of the Mandible of the Rat Following Bilateral Resection of the Mandibular Condyles. *Joseph R. Jarabak and John R. Thompson, Northwestern University Dental School, Chicago, Ill.* The purpose of this investigation was to determine how much condylar growth contributes to the growth of the mandible. A series of measurements were made upon serial cephalometric roentgenograms over the growth period of the rat following bilateral resection in accordance with the technique previously reported by Jarabak and Thompson. Ramus height could not be appraised because the superior extremity of the ramus, the condyle, had been removed. The length of the body of the mandible was determined from the distal spine of the angle, posteriorly, to the superior alveolar crest of the lower central incisor. This measurement was not affected by the resection. The height of the ramus from the coronoid tip to the angle of the mandible was greater by approximately 2 mm. in the resected mandibles of the fully grown rats as compared with their litter mate controls. The notch along the inferior border of the mandible increased in depth 2 mm. over that of the control. The observations indicate that, although there may be local changes in the configuration of the mandible, the overall measurements of the body length and ramus height are not perceptibly altered by the resection of the condyles of the rat.

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88. Elevation of Serum Mucoprotein by Parathyroid Extract. *Milton B. Engel, University of Illinois, College of Dentistry, Chicago, Ill.* There is a relation between the resorption of the glycoprotein containing ground substance of bone and cartilage and the blood mucoprotein level following the administration of small and large doses of parathyroid extract. Young rats receiving 10 to 1,600 units of parathormone were killed at 1 to 96 hours. Blood was withdrawn for mucoprotein determinations. Thin pieces of knee joints and kidney were fixed by freezing-drying for histochemical and morphological studies. Microscopic evidence of accelerated cartilage and bone resorption was present in animals receiving over 500 units. Some bone spicules stained more intensely; others were reduced to fibrillar fragments. Macrophages in the primary spongiosa contained greater numbers of glycoprotein granules and insoluble phosphate and/or carbonate. These changes are interpreted as evidence of breakdown of ground substance. Normal serum mucoprotein levels, expressed in terms of a mannose-galactose standard, and tyrosine, were: carbohydrate 15.9

± 0.8 (S. E. of mean) mg. per 100 c.c., tyrosine 4.1 ± 0.2 mg. per 100 c.c. Eighteen hours after 10 to 30 units of parathormone the values were elevated to around 25 and 6 respectively. Massive doses (800 to 1,000 units in 72 hours) produced values as high as 73 and 21 respectively. Intermediate dosage led to intermediate mucoprotein levels. Anuria and gross kidney changes developed in rats receiving over 1,000 units. In sections, large aggregates of glycoprotein granules were observed in the tubule cells. The lumina of many tubules held homogeneous carbohydrate-containing material, which was sometimes associated with insoluble phosphates. This carbohydrate-containing material is thought to be derived from the serum mucoprotein.

89. A Preliminary Indication of Collagenase From the Oral Environment. *Genevieve D. Roth, and Hugh I. Myers, School of Dentistry, University of Kansas City, Kansas City, Missouri.* Material was secured from patients in the general dental clinic who showed periodontal involvement. Sterile paper points were inserted into the periodontal pocket. These, with the adherent fluids, were transferred to test tubes containing Noguchi fluid medium, a piece of rat kidney, and collagen test paper. The tubes were incubated anaerobically at 37° C. for three weeks. At this time they were opened and the remaining collagen and kidney were placed on a watch glass for study. One c.c. of azocoll was added to the culture in the test tubes and reincubated aerobically for 72 hours at which time azocoll dissolution was noted. Sixty-nine tests were made. The collagen paper was totally destroyed in 15 tests, partially destroyed in 11, and showed no change in 43. The kidney disintegrated in eight, partially in 20, and showed no change in 41. A histological section of the kidney, in a test where collagen was dissolved, showed intact kidney tubules but total destruction of supporting connective tissue stroma. The kidney, in a test where collagen was intact, showed an anemic infarct condition. The azocoll was destroyed in 7, partially in 28, and unchanged in 30. In 9 controls, one became contaminated and showed kidney and collagen paper destruction but the other 8 showed no changes. Those tested with azocoll showed complete destruction in 3 and partial lysis in 5—this action is unexplained.

90. Collagenase-Like Activity in a Salivary Fraction. *Leo M. Sreebny, and M. B. Engel, University of Illinois, College of Dentistry, Chicago, Ill.* Paraffin stimulated, pooled, whole saliva was incubated at 37° C. for 72 hours, and subsequently lyophilized (Fraction No. 1). A portion of the powdered residue was dissolved in distilled water and filtered, in the cold, through a Pyrex "UF" fritted glass funnel. The filtrate was dialyzed against distilled water for 48 hours. Both the dialysate (Fraction No. 2) and the nondiffusible portion (Fraction No. 3) were collected, centrifuged, and lyophilized. The salivary fractions thus obtained were tested for depolymerizing activity with azocoll, a diazotized dye hide powder preparation which liberates a red color when treated with collagenase. This reaction is believed to occur as a result of the depolymerizing effect of this enzyme on the glycoprotein components of the ground substance contained in the hide powder. The azocoll was added to a series of tubes, one of which contained whole saliva, while the others held the different salivary fractions, dissolved in M/15 phosphate buffer, pH 7.0 with Merthiolate (1:10,000). The tubes were agitated for 24 hours at 37° C. Liberation of the red dye was noted after about three hours of incubation in the tubes containing the whole saliva and the lyophilized-dialyzed fraction, indicating the actions of a collagenase-like enzyme. The color became more intense during 24 hours. The absence of color formation in the tube containing the undialized-lyophilized saliva, suggested the presence of an enzyme inhibitor(s) in the dialysate. The presence of such a mechanism was confirmed by incubating the azocoll-lyophil-

ized-dialyzed mixture with a series of concentrations (.001–3 per cent of the dialysate). The resultant color changes revealed a graded inhibitory effect ranging from almost "complete inhibition" (3 per cent) to "no inhibition" in the more dilute solutions (0.1-0.5 per cent).

91. Effect of X-Radiation on Lysozyme Activity of Saliva. *M. G. Wheatcroft, and J. L. Nemes, Naval Medical Research Institute, Bethesda, Md.* A series of saliva samples (pilocarpine stimulated) were collected from 4 male dogs of approximately 25 pounds weight, before and after exposure to x-radiation dosages of 200, 400 and 600 r (total body). The lysozyme activity of the samples remained constant over a period of 3 months, pre- and postradiation, and was unchanged when the total blood and salivary white cell counts decreased to zero. The lysozyme activity was not affected by the varying amounts of saliva produced. Dogs receiving 400 r and 600 r developed hemorrhagic gingivae and large necrotic mouth lesions of tongue and cheek with associated increase of bacterial organisms. Since the lytic property remained high, even with dogs in moribund state, and in the presence of gross mouth infections, the question of the actual purpose and importance of this defense mechanism is further complicated.

92. Studies on the Defense Mechanisms of the Mouth. *Norman S. Simmons, School of Medicine and Dentistry, Rochester, N. Y.* Egg white lysozyme forms insoluble salts with acidic mucopolysaccharides at the pH values found in saliva. If one of the antibacterial factors in saliva is similar to egg white lysozyme, the possibility exists that it may complex with the contained salivary mucins and be removed from saliva as an insoluble salt. To test this possibility the mucins were precipitated from whole saliva (with a quaternary ammonium salt) and the supernatant fluid tested for lytic activity against the test organisms *Sarcinae luteae*. The precipitation of the mucins resulted in a 5- to 10-fold increase in lytic activity of the supernatant saliva as against the control untreated saliva. Saliva previously centrifuged and the quaternary ammonium salt added to the supernatant showed no increase in activity. It may be concluded then that one of the salivary bacteriolytic agents is in salt complex with the contained mucins and can be displaced by a quaternary ammonium salt.

93. Intermediary Carbohydrate Metabolism in Mixed Human Saliva. *B. F. Gurney and G. W. Rapp, Loyola University School of Dentistry, Chicago, Ill.* Carbohydrate metabolism associated with caries formation has been investigated minutely. The present emphasis is not so much on the final end products, such as lactic and other organic acids, but more on the intermediary products, such as the triose phosphates and three-carbon acids other than pyruvic and lactic acid. Since the metabolic reactions are a chain of intermediate steps, the rate and extent of each step is important. The present work shows a difference in the rates and extents of intermediary metabolism found in mixed saliva of caries susceptible and caries immune subjects.

94. Effect of Various Salts on the Production of Acid by Saliva Containing Fermentable Sugars. *J. C. Forbes, and J. D. Smith, Department of Biochemistry, Medical College of Virginia, Richmond, Va.* Inorganic and various organic salts of copper and nickel, such as those of fatty acids, and their complex salts with bases such as ammonia and the ethanolamines, have been found to exert a marked inhibitory action on the production of acid by saliva containing either glucose or sucrose. Concentrations of these metals as low as 0.5 mg. per 100 c.c. exert definite inhibition, while concentrations of 3 to 4 mg. completely prevent acid formation. Concentrations around 10 mg. per 100 c.c. seem

to destroy all the acidogenic organisms in saliva. Inorganic salts of mercury (ic), gold (ous), and silver are also active, whereas the salts of platinum, palladium, chromium, zinc, cobalt, aluminum, magnesium, iron and manganese have little or no activity. Since nickel and copper have relatively low toxicities, a study of their possible use in dentifrices would seem justified.

95. The Effect of Ammoniated Dentifrices on the pH and Acid Producing Capacity of Dental Plaques. *Marguerite F. Little, Finn Brudevold, and Richard Taylor, Eastman Dental Dispensary, Rochester, N. Y.* Preliminary studies of saliva from persons using an ammoniated or nonammoniated dentifrice showed the ammoniated dentifrice to be ineffective in changing the salivary pH and fermentation. Studies were also done in the dental plaque, using an antimony electrode. Following a 10 per cent sucrose rinse, pH measurements were taken at intervals over a period of 1 hour in 55 persons who had been using ammoniated or nonammoniated dentifrices for at least 6 months. The groups showed no difference in the fall of the pH. A rinse of either a high urea ammonia-containing dentifrice or a nonammoniated alkaline dentifrice (5 Gm. suspended in 20 c.c. of water) taken 25 minutes before the sugar rinse decreased the fall in the pH between 30 to 40 per cent. Two low urea containing dentifrices and a nonammoniated neutral dentifrice did not effect the fall in pH after the sugar rinse. A rinse with any one of the ammoniated dentifrices and with the nonammoniated alkaline dentifrice caused an immediate rise in pH. There was a decrease over the next 30 to 40 minutes. The alkaline effect was more prolonged after the high urea ammonia dentifrice than after the other dentifrices. A sugar rinse taken 1 hour before the dentifrice rinse caused an appreciable decrease in the alkaline effect of the dentifrice rinse.

96. Acid Production in Different Mouths by Five Foods. *Hyman J. V. Goldberg, and Basil C. Bibby Eastman Dental Dispensary, Rochester, N. Y.* Persistence of acid production from 5 foods (sprayed cracker, white bread, cookie, coconut candy, and toasted bread) were each tested in 11 student dental hygienists. The purpose of the study was to correlate the persistence of acid production in the oral cavity of various foods with the "decalcification potential" of the food. Subjects brushed teeth, using tap water, and stimulated saliva samples were taken before chewing and at 0, 5, 10, 20, 30, 60, and 120 minutes after chewing. pH and acid production readings were made immediately and after 4 hours incubation at 37.5° C. Results indicated that in incubated samples all of the foods increase the acid production for up to 30 minutes. Because individual variation within each patient group was too great, no correlation could be made with the "decalcification potential" of the foods used.

97. Antibiotic Levels in Human Saliva. *Frederick W. Kraus, Tufts College Dental School, Boston, Mass.* Twenty dental students were each given 3 grams of aureomycin HCl in capsules. Five hundred milligrams were administered every 4 hours. Blood and unstimulated saliva were collected and assayed 2½ hours after both the first and the final dose, and again 24 hours later. The respective mean plasma levels were: 0.63 mcg. per milliliter (Range 0.25-2.0); 4.3 (1.0-16.0); 0.11 (<0.063-0.25). The mean saliva levels were: 0.09 (<0.063-0.25); 0.45 (<0.25-2.0); 0.09 (<0.063-0.25). These results were checked by testing inhibition of test organism (*B. cereus*) in plain saliva and plain serum (3 tests for each, negative) and for alteration of antibiotic activity in saliva or serum under routine assay conditions (2 tests each, negative). Since the experimental readings were clearly attributable to presence of anti-

biotic in saliva, its source was investigated by catheterizing parotid and submaxillary ducts of 4 medicated subjects. The pooled parotid salivas and the pooled submaxillary salivas each contained detectable concentrations of aureomycin. Terramycin HCl similarly administered to 6 subjects was assayed in both plasma and saliva. One 15 mg. aureomycin troche was dissolved in the mouth of each of 6 subjects and, while the mean salivary concentration declined rapidly within the first 30 minutes, it was still, after 3 hours, equal to almost 100 times the highest mean salivary concentration of the systemically administered antibiotic. However, both the excreted and the dissolved antibiotic in saliva were comparable in their reduction of oral bacterial population (8 subjects).

98. Studies of Chromatolysis in the Cat Gasserian Ganglion Following Extirpation of the Pulp and Removal of the Teeth. *Joseph A. Gibilisco, School of Dentistry, Creighton University, Omaha, Neb.* Removal of the peripheral nerve endings from the pulp results in peculiar changes within the cell bodies (neurons) of the Gasserian ganglion. The Nissl substance within these cells undergoes fragmentation or dissolution, and this phenomenon is referred to as chromatolysis. Nine cats were used in this study. The complete right quadrant was operated in each case; the left side reserved for control. The ganglia were removed from 8 to 13 days following operation, and the cells were stained. Normal cells, as well as those demonstrating mild and severe chromatolysis, were recorded. Some chromatolysis was observed in the control ganglia. This is a normal entity. Cells from the control ganglia showed 74.3 per cent normal; 25.5 per cent in the state of mild chromatolysis and 2 per cent severely affected. The operated ganglia showed 69.3 per cent normal; 30.1 per cent mildly chromatolytic; and 5 per cent in severe chromatolysis. A slight but significant variation, when the vast area innervated by the trigerminal nerve is considered. The animals sacrificed at 8 days showed more chromatolysis than those sacrificed at 13 days. There is a steady decline in "atypical neurons" observed in the interval of time from these two extremes.

99. Clinical Test of an Ammoniated Dentifrice. *Roland R. Hawes, Eastman Dental Dispensary, Rochester, N. Y.* A one year study of the caries incidence was made on groups of school children using a control and test dentifrice containing 8 per cent urea. Both groups of children brushed their teeth under supervision at the beginning of each school day and were given supplies of brushes and dentifrice for home use. All examinations included bite-wing radiographs. Intermediate evaluations of brushing procedure and caries incidence were made. Additional data concerning the incidence of malocclusion and gingivitis were also recorded.

XI. Dental Materials Section, Abstracts 100-113

100. An Estimate of the Fit of 100 Acrylic Denture Bases. *E. W. Skinner, and E. N. Cooper, Northwestern University School of Dentistry, Chicago, Ill.* One hundred maxillary acrylic resin denture bases were prepared on duplicate stone casts. The same acrylic resin and curing cycle were used throughout, but the conditions of mold lining were varied. The fit of the base as judged by the posterior area was estimated by three different observers. It was found that the fit of the base was better when the cast was tinfoiled rather than when a substitute was used, regardless of how the remainder of the mold surface was treated. The effect of each mold lining on the fit was observed. Eight different mold liners were employed.

101. The Influence of Rotational Speed on Temperature Rise During Cavity Preparation. *R. C. Vaughn, and F. A. Peyton, University of Michigan School of Dentistry, Ann Arbor, Mich.* This is a report of an investigation of the temperature rise which occurs from cutting tooth tissue with steel burs and tungsten carbide burs when operated at speeds up to 11,000 r.p.m. Values from only the fissure type burs are reported. Operating pressures of from ½ to 2 pounds were employed in the tests. Conventional dental equipment was used in this study for cutting extracted teeth. The temperature change was measured in the tooth at various distances from the bur and in the area immediately adjacent to the bur. Graphs of the temperature change as a function of speed and pressure are presented. In this study it was observed that the maximum temperature was reached within a few seconds from the start of the cutting operation when intermittent pressure was used. The maximum temperature of 160° F. was observed when operating at 11,000 r.p.m. and 2 pounds pressure.

102. Cutting Efficiency of Dental Burs Utilizing a Straight Handpiece. *E. E. Henry, and F. A. Peyton, University of Michigan School of Dentistry, Ann Arbor, Mich.* The ideal cutting instrument for restorative dentistry has been described as one which will remove the most material with the least expenditure of energy, the least vibration, and the least heat introduction, while having the longest life. The present study of dental bur cutting efficiency shows the amount of material removed as a function of pressure (¼ lb. to 1.65 lbs.) and speed (from low values to 8,000 r.p.m.). From this data is developed a graphical three dimensional surface with the above quantities as coordinates. The torque and energy required for the operation of the handpiece, alone and during cutting operations, is also shown; the data for the above are obtained through the use of a motor which has been electromechanically calibrated. These data have been gathered for the No. 557 and No. 558 fissure burs, and the No. 37 and No. 39 inverted cone burs. Data has also been collected substantiating previous recommendations that diamond points and stones are generally not indicated for use on soft materials.

103. The Chemistry of Self-Curing Resins. *G. M. Brauer, National Bureau of Standards, Washington, D. C.* The most outstanding development in dental materials in the last few years is the use of resins which will polymerize at room temperature to a hard esthetic material. The synthesis of the monomer, mechanism of polymerization, history of the development of these self-curing resins, and structure of the products are discussed. Self-curing resins are obtained by polymerizing a methyl methacrylate monomer powder mixture containing an organic peroxide and amine accelerator. In order to obtain the best results in the polymerization, the monomer-catalyst-accelerator ratio used is important. The optimum ratio will vary for different catalyst-accelerator systems. The presence of the promoter speeds up the decomposition of the organic peroxide so that enough free radicals are formed to initiate the rapid polymerization of monomer at room temperature. Tertiary amines such as dimethyl-meta-toluidine are satisfactory promoters for dental resins due to their high reactivity. Catalyst and accelerator fragments are built into the resin and their presence will affect somewhat the properties of the polymer. Self-curing resins used at present will show fair color stability and their mechanical properties are not equal to those of resins polymerized at higher temperatures.

104. Significant Physical Properties. *G. C. Paffenbarger, National Bureau of Standards, Washington, D. C.* The most important physical properties that have an unusual dental significance are in the order of their possible

criticalness; shrinkage during polymerization, thermal expansion, strength, soundness, and color stability. The degree of polymerization, that is, the proportion of unpolymerized monomer remaining adversely affects most of the foregoing properties. The shrinkage of the resin during polymerization and its high coefficient of thermal expansion constitute by far the most serious defects in the direct resinous filling materials. The main advantages are the extreme insolubility in the mouth environment, low thermal conductivity, unusually high esthetic qualities and comparative blandness to the pulp tissues. The shrinkage during polymerization may be reduced and forced to take a directional path. The reduction may be accomplished by using the minimum amount of monomer in the mix, by delaying the placement of the resin in the cavity for as long as possible, or by building the restoration step by step. The shrinkage may be forced to concentrate in sections of the cavity farthest from the margins by proper placement of undercuts and by keeping the matrix strip unremittingly taut until the initial phase of the polymerization is completed. The thermal expansion may not be meaningly changed so the patient should be instructed to protect the resin restoration from extremes of temperature. The comparatively low strength, low hardness, low modulus of elasticity, and high flow limit the resin to sheltered and well protected cavities. Most of the porosity of the resin restorations is caused by the incorporation of air during mixing. The not-too-good color fastness to light does not seem to be an important clinical problem.

105. Evaluation of the Auto-Polymer Direct Plastic Filling Materials. *E. Edward Kraus, and Leland L. Kraus, Marquette University, Milwaukee, Wis., and Medical College of Virginia, Richmond, Va.* The new, and very controversial material, auto-polymer plastic filling material, does not adequately protect teeth when used as a direct filling because: (1) It does not hermetically seal by compression. (2) It does not hermetically seal by virtue of cementation. (3) The always present chemical shrinkage can never be compensated by expansion due to water absorption. (4) It percolates mouth fluid through the margins, due to its great difference of thermal-dimensional change compared with tooth structure. (5) It is not bacterio- or cario-static in the presence of mouth fluids. (6) Technic cannot overcome its shortcomings. (7) The presently available auto-polymers are basically identical. (8) All cases exhibited a peculiar softening of the cavo-surface. Our clinical findings are based on a study of over 500 cases, from various operators, dating from February, 1947. The bacteriological study was based on approximately 50 clinically acceptable cases. The specimens were obtained under the most sterile conditions possible. Specimens were incubated 24 to 48 hours in nutrient broth or nutrient and agar slants stab cultures. There was a universal finding of oral bacteria in all cases and areas. Putrid odor was observed in all cultures. Auto-polymers fail to protect teeth when they are used as direct filling materials. Auto-polymers may be used in proper areas as a restoration when formed in the cavity and recemented with zinc phosphate or silicious cement.

106. Pulpal Response to the Insertion of the Self-Curing Resins. *Alexander Seelig, Montifiore Hospital, New York, N. Y.* Two specific plans were followed: The first was the testing of the product Fasterown Filling Plastic bought on the open market in February of 1949. Under nembutal anesthesia 55 teeth of 3 young Rhesus monkeys were used to prepare 87 cavity preparations in which various types of fillings were used. The cavity preparations were then filled with commonly used filling materials as well as with plastic fillings. The plastic fillings were put in deep and shallow preparations. They

were put in dry as well as wet, also with or without a liner. Histological sections were made after the animals were sacrificed in periods of 1, 4 and 7 days. From this study it was concluded that no matter how the material was mixed nor how deep or extensive the cavity preparation no pupal pathosis will follow the placing of this plastic filling material. The second study was carried out in the same way as the first except that 4 other acrylic filling materials were also tested. The findings indicated that not all the acrylics are the same in their reaction upon the pulp. The conclusions that can be drawn depend upon the brand of filling material that is under discussion. There are two types: those that irritate the pulp and those that do not.

107. Thermal Conductivity of Dental Materials. *W. G. Simeral, University of Michigan School of Dentistry, Ann Arbor, Mich.* In the pertinent literature there is only a limited amount of information concerning the thermal conductivity of dental materials other than metals. Similarly, little is known about the absolute thermal conductivity of tooth structure itself. This report describes an apparatus designed specifically to measure the thermal conductivity of nonmetallic restorative materials such as the cements and resins currently used in the dental profession. Measurements with an accuracy of from 2 to 3 per cent have been made on several types of cements and resins as well as on glass and quartz. These last served as checks on the equipment. The design of the apparatus permits measurement of samples prepared as discs 2 centimeters in diameter and from 1 to 3 millimeters in thickness. Additional apparatus has been designed, as an attachment to the present equipment, which will make possible the measurement of the thermal conductivity of sections 3 to 5 millimeters in diameter.

108. Physical Properties of Amalgam as Influenced by Variation in the Surface Area Per Unit Weight of the Alloy Particles. *W. S. Crowell, S. S. White Company, Philadelphia, Pa., and R. W. Phillips, Indiana University School of Dentistry, Indianapolis, Ind.* The object of this research was to show the effect of the surface area of the particle on the physical properties of amalgam. Special ingots were poured and particles cut in a manner which would minimize variation in properties due to the location in the ingot. Four different size particles were prepared, each with a known surface area. Results show the setting expansion and setting time to vary regularly with the increase in surface area. Expansion appears to be a linear function of surface area. The smaller the area of the particle, and thus the greater the surface area per gram of alloy, the greater the one hour compressive strength. The twenty-four-hour strength also increases slightly with a decrease in particle size. Flow was constant on all sizes except for the one having the smallest surface area. Data is presented showing variation in properties when different ingots of the same chemical composition are tested as well as cuttings from different parts of the same ingot.

109. Cooperative Comparative Testing of Dental Gold Alloys. *R. C. Brumfield, J. F. Jelenko Company, New York, N. Y.* This paper is a progress report on comparative testing of dental gold alloys by laboratories of member firms of the Dental Gold Institute. Research representatives of these firms constitute a standing committee of this Institute. This committee has recently adopted new and revised methods of testing dental gold alloys. Member laboratories have engaged in comparative tests as a check on some of the testing methods described, as well as on equipment and procedures used in testing by the various laboratories. These tests include yield strength; ultimate strength;

elongation; and modulus of elasticity of wire; as well as upper and lower limits of the melting range of casting golds. Results are compared and discrepancies are discussed and explained.

110. The Effect of Section Size on the Mechanical Properties of Wrought Gold Wires. *S. H. Bush, University of Michigan School of Dentistry, Ann Arbor, Mich.* Three dental golds, varying widely in chemical composition and in microstructure, were selected to determine the effect of section size on the mechanical properties of these wires. Four section sizes, 20 gauge (0.030 inch), 18 gauge (0.040 inch), 16 gauge (0.050 inch), and 14 gauge (0.064 inch), were investigated; these wires were tested both in bending and in tension, using the proportional limits as a criterion for comparison. The wires were tested in the softened and the hardened state. One wire was also tested after heating to 1700-1800° F. (927-982° C.) for one hour to determine the effect of grain size on the mechanical properties. No definite correlation was observed between section size, as such, and proportional limit. Results indicated that grain size and, possibly, chemical composition were the important factors. On comparing the proportional limits of the alloys as a function of section size it was observed that the values varied to the same extent in both the softened and hardened state. Both bend and tensile tests proved to be equally satisfactory when used as a medium of comparison between section size and proportional limit. In some cases a definite difference in proportional limit was observed below a critical section size. Above this critical section size no such difference existed. In specimens heated to a higher temperature this critical size was shifted to a larger cross-section. Since a similar change in grain size was observed, it was concluded that grain size was one of the most important, if not the sole controlling factor in determining the critical section size of the wire, which must be used to determine the true value of the proportional limit.

111. Change of Articulation After Processing of Complete Denture. *D. B. Mahler, University of Michigan School of Dentistry, Ann Arbor, Mich.* In the processing of complete dentures, using the compression flasking technique, a problem exists which prevents the exact reproduction of the occlusion as set up on the waxed-up denture. This difficulty is a result of a shifting of the teeth (both horizontally and vertically) caused by forces exerted during processing. This paper involves a quantitative determination of the amount of vertical shifting of the teeth due to pressures exerted by the acrylic resin before polymerization has taken place. It was found that this vertical shift is a function of the material used in the "splint" or "third pour" as well as the pressure in the resin caused by the packing procedure. A method of determining the pressure in the resin as a result of the flasking process is also described. Recommendations are made which will yield a minimum amount of vertical tooth movement caused by this pressure.

112. A Method for Evaluating Adaptation of Restorations. *R. B. Wolcott, and R. J. Nelsen, National Bureau of Standards, Washington, D. C.* It was observed that most restorations, regardless of their apparent exactness and marginal adaptability, possess an unusual defect in the seepage of fluid from their margins when subjected to temperature variations. This phenomenon is believed to be caused by (1) a difference in the coefficients of thermal expansion of the tooth structure and the restorative materials, and (2) thermal expansion of water or gas present in the interfacial pathways. This mechanism of thermal seepage may (1) explain the development of certain caries processes, (2) help to evaluate the effectiveness of restorations as a cavity seal, and (3)

provide another explanation for the development of hypersensitivity which is associated with some types of temporary and permanent filling materials.

113. Chemical Bonding of Plastic Teeth to Denture Bases. *T. E. Fischer, and A. F. Serio, National Bureau of Standards, Washington, D. C.* Many users of teeth indicated that some plastic teeth did not bind satisfactorily to the base. This report will give the results of an investigation of the chemical bonding of teeth to denture bases. The method developed for testing the chemical binding is to cure a cylindrical section of tooth in a rod of denture resin, and to measure the force necessary to break the union. Results on seven brands of the presently available plastic teeth were obtained. The following conclusions may be made from these observations: (1) Presence of a wax on the teeth, caused by incomplete removal, will definitely weaken the bond between teeth and denture base resin (2) Foil substitutes also cause a poor bond if not completely removed (3) Wax is not satisfactorily removed by the application of hot water alone (4) Satisfactory chemical union was obtained for both craze resistant and craze susceptible teeth when the wax was completely eliminated from the binding surfaces (5) A practical method for removal of wax from dentures processed by the normal technique is by the use of a hot detergent solution (one teaspoonful per pint of water) (6) Results on two brands of low temperature or self-curing denture base indicated that they did not properly bond to acrylic teeth.

XII. Abstracts Read by Title: 114-169

114. The Postnatal Growth of the Maxilla in Macaca Mulatta. *Louis J. Baume, Division of Dental Medicine, College of Dentistry, George Williams Hooper Foundation for Medical Research, University of California, San Francisco, Calif.* The upper jaws of 41 macaque monkeys were subjected to biometric and roentgenographic evaluation. Twenty were studied histologically. The growth of the maxilla was characterized by coordinated activity of sutural and periosteal osteogenesis. The main centers of longitudinal development were situated at the maxillary tuberosity and the premaxilla. Both periosteal as well as sutural ossification at these zones contributed equally to increase in the length of the maxilla. An identical mechanism at the facial surface and median palatal suture was instrumental in growth in width. Vertical development was brought about by combined mechanism of sutural and periosteal bone formation at the orbits and the palate respectively, but only to a small extent at the alveolar crests and cribiform plates. Contrary to opposing contentions, no specific preponderance was evidenced in the contribution of the sutures or the periosteum to facial bone growth. Developmental processes (apposition and resorption) at various surfaces seemed to be governed by the specific growth pattern of the neighboring organ (eye, tongue, etc.), rather than a local autonomy of the osseous growth site itself. The diagramatic conception of rigidity of the developmental pattern was refuted by findings of the following changes in facial proportions: a) Decreasing profile angle was due to greater forward growth of the alveolar portion in relation to the orbito-nasal portion of the maxilla (or relative stability of the orbits). b) Increase in nasal height was concomitant with decrease in anterior alveolar height due to resorption of the labial plate after replacement of incisors. This was associated with an altered angulation of permanent incisors in comparison to their predecessors and a changed course of the alveolar plane in relation to the floor of the nose. c) There was an overall increase in all portions of maxillary bone in contrast to stability of the pyramidal process of palatine bone and of the pterygoid process of sphenoid bone. Factual

evidence thus favors the old concept of Martin that an ontogenetic change of facial proportions determines development of the face.

115. The Postnatal Growth of the Mandible in Macaca Mulatta. *Louis J. Baume, Division of Dental Medicine, College of Dentistry, George Williams Hooper Foundation for Medical Research, University of California, San Francisco, Calif.* Development of the mandible was studied biometrically and roentgenographically in 41 Rhesus monkeys representing consecutive dental developmental stages during period between fully formed deciduous dentition and permanent dentition. Twenty were selected for histologic study in 3 different planes. Biometric, roentgenographic and histologic data lead to the following conclusions: Growth of the mandible during the periods studied occurred on every surface. There was considerable forward growth at the anterior surface of the mandibular body; osteogenic activity at its lower border contributed a quarter of the gain in mandibular body height. This indicates that both sites are not qualified to serve as fixed planes in the roentgenographic superposing technique. Relatively stable regions due to osteogenic aplasia were found at the mandibular canal below the second premolar. Histologically the alveolar process of permanent dentition was entirely new formation formed during eruption of each permanent tooth and, for the main part, at the expense of the former mandibular body. Development was characterized by a small amount (2.9 average) of vertical alveolar growth in the premolar region and even none in the anterior region. The finding of 6.5 mm. increase in intercondylar width and 12.1 mm. in overall mandibular length together with histologic evidence indicated that the mandibular ramus was subjected to a growth movement in both lateral and dorsal directions, thus producing continuously an enlarged replica of the former mandible. Traditional rationale of mandibular growth, namely, that posterior border represents the site of most active periosteal growth was based only on studies of the longitudinal aspect. In this investigation the functions of membranous bone formation, resorption and apposition at the sites of muscle insertions, was found to be performed by tendinous perimysia in place of distinct periosteum. This indicates osteogenic property of muscle tissue. Endochondral growth center at the condylar head proved to be the pacemaker and organizer of mandibular growth although a relatively small increase in ramus height (4.7 mm.) was typical for Macaca. Chondrogenesis proceeded in a dorsolateral direction with a very low vertical component. Contrary to present concepts a small increase in ramal, as well as dental, height was conducive to a uniform maintenance of the end-to-end occlusion in transient deciduous and permanent dentitions.

116. The Relationship Between Jaw Growth, Tooth Development and Tooth Eruption. *Louis J. Baume, Division of Dental Medicine, College of Dentistry, and the George Williams Hooper Foundation for Medical Research, University of California, San Francisco, Calif.* Histologic study of entire jaws of 20 Rhesus monkeys in 3 planes at different developmental stages of dentition disclosed the following biogenetic relationship of jaw growth to tooth development: It was passive and reactive. "Passivism" was expressed by uniformity of bone resorption at inside of crypts during expansional growth of tooth germs. This initiated early root resorption at the lingual surface of the predecessor. Continuous osteoclasia at the fundus of the germs did not confirm the assertion that position of the Hertwig's sheath remains fixed during tooth development. Reactive bone formation due to odontogenesis was evidenced by the following phenomena: (a) Pre-eruptive movements of the germ induced lamellar apposition in certain areas of the crypts; (b) Spacing of the anterior predecessors

was associated with formation of a bony septum between the already eroded lingual root surface of the predecessor and its succeeding germ; (c) Apposition at labial plates of the anterior deciduous teeth prevented their early exfoliation; (d) Development of lingual mandibular bulk was concomittant with pre-eruptive movement of accessional molar buds and produced the lingual plate of the future alveolar process of accessional mandibular teeth. (e) Mesial migration of teeth in function which was distinct in the mandibular arch was interpreted as a reactive growth movement of the anterior alveolar portion to development of accessional teeth in the posterior region. The complicated path of mandibular molar buds revealed the inadequacy of present concepts of the mechanism of eruption. Pre-eruptive movements of a lower permanent molar comprised a lingual drifting and rotation and a distal downward tilt; active vertical eruption consisted in ¾ downward movement combined only in the second to last phase with an upward movement of the crown. It was terminated by a downward growth of the roots into the mandibular body. Universal observation of maintenance of epithelial and fibrous tissue connection of tooth germ with oral mucosa by gubernaculum and proliferation of follicular epithelium at cessation of amelogenesis and initiation of eruption suggest the application of the laws of experimental embryology (Harrison and Spemann) to interpretation of the eruption process. The dental organ is thought to act as "organizer" upon the connective tissue environment, its epithelium being provided with an "organogenic," as well as "topogenic," faculty. When this determination is accomplished, follicular epithelium was seen to be replaced by oral epithelium forming a new attachment to the tooth.

117. The Effectiveness of Ammoniated Dentifrices in Reducing Oral Lactobacillus Counts. *Basil G. Bibby, and Thomas A. Nevin, Eastman Dental Dispensary, Rochester, N. Y.* This study was carried out to determine whether or not the use of ammoniated dentifrices would reduce oral lactobacillus counts as has been claimed by various investigators and numerous advertisers. In order to establish an experimental basis for the effectiveness of NH_4 + compounds against oral organisms, in vitro tests were carried out, through the use of a cup-plate method by which various NH_4+ compounds and dentifrices were checked for their ability to prevent acid formation in carbohydrates. Controls were run simultaneously. No essential differences were observed. In vivo testing was carried out, using as subjects the 75 young women of the student body of the School for Dental Hygienists. The subjects were divided into 3 comparable groups on the basis of caries incidence. Group I used an ammoniated tooth paste exclusively. Group II used standard nonammoniated tooth paste exclusively. Group III was instructed to continue the use of usual dental habits, except that on the days when samples were collected, each individual would refrain from brushing her teeth. Four salivary samples were collected from each individual during a test day which occurred one day each week for 7 weeks. The specimens were plated on tomato juice agar, according to the method of Jay and Arnold, and incubated for 4 days. Simultaneously, selected specimens were subjected to total acid titrations, and the capacity to ferment carbohydrates rapidly, measured against a glass electrode. After a period of 7 months, the groups were again sampled, and the processes repeated. Evidence was obtained to show that NH_4+ containing dentifrices do not produce any significant reduction in oral lactobacillus counts, where either a short term study, or a long term study is considered.

118. Use of Aqueous Urea Solution in the Field of Endodontia: Preliminary Report. *Harry Blechman, and Morris Cohen, College of Dentistry, New York University, New York, N. Y.* Medical literature on urea and urea

combinations is very extensive and has presented many useful clinical applications. This work has been extended to the field of endodontia. A 30 per cent urea solution has been found to be an excellent root canal wash, following vital pulp removal with hemorrhage. The solution is nontoxic and nonirritating to the periapical tissues and does not disperse the hemoglobin into the dentinal tubules, to cause tooth discoloration. The 30 per cent solution is also an effective débriding agent for necrotic organic tissue found in the root canals and is an excellent vehicle for antibiotics such as aureomycin and terramycin. Saturated dressings of these compounds have been sealed into root canals for periods of 1 to 10 days, to reduce inflammation and infection, or to prevent infection following pulp canal operation. Bacteriologic root canal cultures and bio-assay of the medicated cotton point dressings after stated periods of confinement in the root canals were performed. Of 15 clinical cases it is concluded that a 30 per cent urea solution is an excellent root canal flush, and is an effective débriding agent for necrotic tissue remnants found in root canals. The solution in combination with either aureomycin or terramycin shows marked antibacterial properties when used as a dressing in canals.

119. A Cephalometric Radiographic Study of a Correlation of the Occlusal Vertical Dimension and the Rest Vertical Dimension of the Face in Various Age Groups of Individuals. *Philip J. Bright, and John R. Thompson, Northwestern University Dental School, Chicago, Ill.* Arithmetic means were established for the size of the interocclusal clearance in 2 age groups of individuals possessing excellent occlusions. They were determined as follows: Mixed dentition group 4.24 mm., young adult group 3.31 mm. The comparison of the means of the above age groups indicates that there is a significant difference in the size of the interocclusal clearance between these dissimilar groups. The comparison of a previous investigation of the mixed dentition with the mixed dentition group of this study indicates that no difference in the size of the interocclusal clearance exists between these similar age groups. The comparison of a previous investigation of an adult group with the young adult group of this study indicates that there is a significant difference in the size of the interocclusal clearance between these dissimilar age groups. There was no significant difference between the 3 registrations of the rest position for the mixed dentition group and for the young adult group. The 3 registrations of the rest position were quite consistent for each individual.

120. Diagnosis and Therapy of Dental Foci and Arthritis in Focal Infection. *Fritz Driak, Vienna, Austria.* Tonsils, teeth, and their surrounding structures frequently lead to focal infections. Diagnosis is often difficult. It entails a systematic examination (roentgenograms, pulp testing, etc.). The Viennese school rejects provocative methods with the exception of irradiation with small x-ray doses of 1-5 r (Driak and Pape), that constitutes a harmless method of testing. All foci have to be completely removed. Periodontal foci are of secondary importance compared to periapical foci. An exact root canal treatment may be successful, leading to spontaneous healing of foci of infection. Diagnosis and treatment is possible only through cooperation of physician and dentist.

121. A Roentgenographic Study of Edentulous Jaws. *Ralph W. Edwards, University of Kansas Medical Center, Kansas City, Kan.* A study was undertaken to determine to what extent apparently normal edentulous jaws are involved with unerupted teeth, fractured roots, foreign bodies, and pathologic areas. The jaws selected for study had normal mucosa without evidence of

pathologic involvement. If fractured roots were visible through the mucosa or if a single unerupted tooth were present the patient was excluded from the survey. A total of 1,050 individuals were studied, representing two groups of patients. Group A consisted of 800 patients, 362 men and 438 women, with both jaws edentulous. Group B represented 250 patients, 106 men and 144 women, with either the maxilla or mandible edentulous, the other jaw being wholly or partially dentate. Of the 1,050 individuals studied, 677 (64.5 per cent) had jaws that were negative; thirty-five patients (3.3 per cent) had 44 unerupted teeth present; 274 patients (26 per cent) had a total of 392 fractured roots remaining in the jaws. In 28 mandibles fragments of amalgam fillings were found in the premolar and molar regions; in one maxilla a portion of a dental bur was found and in another maxilla a portion of a forceps beak was observed; 9 patients (0.9 per cent) had median anterior maxillary cysts; 13 (1.2 per cent) had radicular cysts; 41 (3.9 per cent) had areas of residual infection remaining after dental extractions.

122. A Study of Tooth Size, Dental Arch Length, and Facial Balance in Cases of Class I Malocclusion. *Irving J. Fleet, and F. T. Barich, Northwestern University Dental School, Chicago, Ill.* The size of the teeth in cases of Class I malocclusion with crowding appear to be slightly larger than average values for tooth size as determined by previous investigators. In both maxilla and mandible no significant correlations between the size of the teeth, nor the length of the arches, and the amount of crowding were obtained. The amount of crowding appears to be a function of the interaction of both variables, the size of the teeth and the length of the arch, as evidenced correlating the ratio of tooth size to arch length with the amount of crowding. Facial balance, as measured by the linear distance of 1 to NP and by the angle of inclination of the central incisors, of 1 to NS, appears to vary inversely with the amount of crowding in the cases studied. The mechanism by which facial balance varies inversely with the amount of crowding in cases of Class I malocclusion has been explained as a partial compensation by the teeth for discrepancies between tooth material and bony support.

123. Radiographic Methods of Analysis of the Structures of the Lower Face to Each Other, and to the Occlusal and Maxillary Planes of the Teeth. *Robert S. Freeman, Dale F. Rasmusson, and T. M. Graber, Northwestern University Dental School, Chicago, Ill.* The purpose of this study was: To test the reliability of making cephalometric appraisals of the lower face as an aid in the evaluation of orthodontic cases and to devise a simple method of taking oriented roentgenograms of the area of the lower face with the dental x-ray machine which may also be readily adaptable to the average orthodontic office. In this study, 170 lateral roentgenograms of individuals with teeth in occlusion were investigated. Emphasis was placed upon the interpretation of the relation of the facial elements to one another. Frankfort Horizontal was used and studied as a plane of reference. Points *A* and *B* were used as representative of the anterior borders of the apical bases. By constructing an angle of $A \times B$, this relationship was measured and recorded in numerical values in the normal and malocclusion groups. Other relationships of the facial elements were investigated. The relation of the anterior apical base line, was also investigated in its various angular relationships to the maxillary, occlusal and mandibular planes. Linear measurements were made to determine incisor and alveolar prognathism and vertical and horizontal overbite. Sixty-eight per cent of the cases within the group investigated fall within the variable range of the arithmetic mean. A portable head-holding device was designed and constructed

to be adaptable to the dental chair and for use with the dental x-ray machine. Satisfactory extra-oral radiographs of the lower face can be taken with a 4″ × 5″ film using this portable head positioner and a dental x-ray machine. The patient can be accurately reoriented for successive radiographs, and the angular and linear measurements (used in this study) made from the 4″ × 5″ radiographs can be compared directly to those from an 8″ to 10″ radiograph. This study produced a technique that is inexpensive to install, economical to operate, requires no additional space, and furnishes the orthodontist with accurate information for diagnostic purposes that will be of equal value to that obtained by present cephalometric methods.

124. A Study of Sutural Facial Growth by Means of Metallic Implants in the Macaca Rhesus. *Benjamin J. Gans and Bernard G. Sarnat, University of Illinois College of Dentistry, Chicago, Ill.* The contribution to the growth of the face of the fronto-maxillary, fronto-zygomatic, zygomatico-maxillary, zygomatico-temporal and premaxillo-maxillary sutures was studied in 8 Macaca rhesus. The suture was exposed surgically and after drilling a cavity, amalgam was inserted in each of the adjacent bones. The distance between 40 such paired implants was measured. At the end of the experimental period (7 to 10 months), the animals were sacrificed and the skulls dissected. Measurements were again made of the distance between the implants on each side of the suture. The amount of separation of the paired implants for a given period was thus determined. The findings revealed that in a 7-month period the increase in the distance between the implants was greatest at the zygomatico-temporal suture (1.7-3.3 mm.) and less at the zygomatico-maxillary suture (1.0-1.4 mm.). The amount of separation between the implants of the fronto-maxillary, fronto-zygomatic and premaxillo-maxillary sutures was so little that it could not be accurately measured. The implants which originally were on the lateral surface of the zygomatic arch and the lateral surface of the lateral wall of the orbit were seen, at the completion of the study, on the medial side. This study demonstrates: (1) bone growth occurs at sutures, (2) rate of bone growth is greatest at the zygomatico-temporal and zygomatico-maxillary sutures of those studied, (3) appositional growth at the site of the implants was greatest on the lateral surface of the zygomatic arch and the lateral surface of the lateral wall of the orbit.

125. A Cephalometric Radiographic Investigation of the Skeletal Pattern of a Specific Dental-Facial Anomaly. *Warren E. Gerber, and John R. Thompson, Northwestern University Dental School, Chicago, Ill.* The following facial skeletal anthropometric measurements differed significantly from those of normal control groups: (a) The angle NS-GoGn was significantly larger in the experimental sample. (b) The angle N-S-Gn was also significantly larger in the experimental sample. (c) The angle S-N-Gn was significantly smaller in the experimental sample. (d) The angle S-N-b was significantly smaller in the experimental sample. (e) The S-N-a, S-N-b difference was significantly larger in the experimental group. (f) The OP and Mand. PL. angles were significantly larger in the experimental sample. There appears to be a positive correlation between the angles NS-GoGn, and Mand. PL. Likewise, a negative correlation seems to exist between the angles NS-GoGn, Mand. PL, and the angle S-N-Gn. Syndrome cases present varying degrees of disturbed facial balance which appear to be directly associated with a large mandibular angle (condylar angle) a retrusiveness of the chin point, and an open bite. The syndrome seems to be associated with an increase of anterior face height and a decrease in posterior face height. Temporomandibular joint disturbance

could not be definitely associated with the syndrome but, since some of the cases experienced a disturbance of joint function, this area should always be considered during functional analysis. With the exception of the open bite the articulated dental casts did not conclusively reveal any characteristic difference between syndrome cases and the average case. No definite hypothesis could be formulated from the case history data or statistical data concerning the morphology or physiology of the syndrome; however, it was considered feasible that the facial pattern of syndrome cases could be the result of either acquired or hereditary growth influences. For convenience of discussion the associated subjective and objective characteristics of the anomaly investigated have been referred to as a syndrome. It is suggested that they be considered and accepted as a syndrome.

126. A Radiographic Study of the Position of the Mandibular Condyle in Individuals Possessing Malocclusion of the Teeth Before and After Orthodontic Treatment. *Chester R. Glowacz, and Vernon R. Boman, Northwestern University Dental School, Chicago, Ill.* The paths of closure of the mandible from rest position to occlusal position that appeared to be normal, disclosed an improvement toward the normal (hinge movement). In most instances detectible amounts of mandibular displacement in Class II, Division I malocclusion cases as shown by temporomandibular joint radiographs either completely disappear or appreciably improve during orthodontic treatment. Orthodontic treatment of Class II, Division I cases (Angle) with a posterior-superior mandibular displacement, does not appreciably affect the occlusal position of the mandibular condyle. Usually there is no apparent change in the rest condylar position during orthodontic treatment of Class II, Division I cases showing normal condylar movement from rest position to occlusal position. In most cases the reduction of mandibular displacement (posterior) during orthodontic treatment is accompanied by a permanent forward repositioning of the occlusal position of the body of the mandible. It is difficult, however, to differentiate between mandibular repositioning and mandibular growth. In most cases the reduction of mandibular displacement during orthodontic treatment is accompanied not by a permanent forward repositioning of the occlusal position of the condyle but rather a change of the rest condylar position. The apparent change in the rest condylar position during orthodontic treatment when it does occur is probably associated with growth changes. The results of this study should not be misinterpreted to imply that "jumping of the bite" or repositioning the mandible anteriorly to its normal position is feasible. Furthermore, it is probable that mandibular repositioning and mandibular growth are interrelated.

127. Changing Philosophies in Cleft Palate Management. *T. M. Graber, Northwestern University Dental School, Chicago, Ill.* Cleft palate individuals as a group show different patterns of maxillary growth, laterally, anteroposteriorly, and vertically. Early and traumatic surgery results in the greatest deformity. There is a positive correlation between the number of operations, the amount of scar tissue, and the degree of insult. To minimize interference with growth centers, it seems advisable to postpone surgical correction at least until the end of the fourth year of life, when five-sixths of the total maxillary width has been accomplished. However, it must be remembered that downward and forward growth, which depends primarily on the sphenooccipital synchondrosis and sutural activity continues until 20 years of age. It appears that the surgically repaired palate grows more slowly than the surrounding soft tissue. This results in increasing functional difficulty and ultimate structural inadequacy. Patients whose palatal clefts have not been

surgically manipulated show a pattern of maxillary growth that is essentially normal. Closure of nonoperated clefts by prosthesis seems quite successful, if judged by the general intelligibility of speech and ease of mastication and deglutition. Rehabilitation requires the coordination in one organization of all the skills and knowledge peculiar to the cleft palate problem. Only by grouping services and correlating information and ideas can each man render the fullest value of his particular field.

128. Protective Effect of Methylene Blue Against the Enamel Depigmentation of Vitamin E-Deficient Rats. *Humberto Granados, and Henrik Dam, Department of Biology, Polytechnic Institute, Copenhagen, Denmark.* The incisor depigmentation in vitamin E-deficient albino rats is influenced by quantitative and qualitative changes in dietary fat, protein and minerals (Granados, Aaes-Jørgensen and Dam: *Brit. J. Nutrition,* 3: 320, 1949, *Acta path. et microbiol. Scandinav.,* 27: 304, 1950, Granados and Dam: *J. D. Res.,* 29: 689, 1950, Dam, Granados and Maltesen: *Acta physiol. Scandinav.,* 21: 124, 1950, Glavind, Aaes-Jørgensen, Granados and Dam: *J. D. Res.,* 29: 689, 1950). A number of redox substances and sulfur compounds protect against several symptoms of vitamin E deficiency in chicks and rats, including the incisor depigmentation (Dam and co-workers: *Acta physiol. Scandinav.,* in press, *Acta pharmacol. et toxicol.,* in press). A good number of experiments have been carried out with weanling rats fed purified rations (containing 20 per cent casein and 20 per cent cod liver oil) without vitamin E, but supplemented with 0.126 per cent methylene blue, and with 0.02 per cent vitamin E (α-tocopherol acetate). The degree of incisor depigmentation in the various groups has been recorded weekly using a method previously described (Granados and Dam: *Odontologisk Tidskrift,* 56: 457, 1948). The results have shown that, at the tested levels, methylene blue protects as tocopherol against enamel depigmentation.

129. Deleterious Consequences of a Mineral Stress as Influenced by Vitamin D and the Parathyroids. *Roy O. Greep, and Clary J. Fischer, Harvard School of Dental Medicine, Boston, Mass.* Intact and parathyroidectomized rats 28 days of age were fed synthetic diets varying widely in calcium and phosphorus composition for a period of 4 weeks. Each calcium:phosphorus ratio selected was fed with and without vitamin D to both categories of animals. The operations were done on the first day of the experiment. Diets deficient in phosphorus had a relatively greater effect on body growth than diets deficient in calcium. Maximal growth however was not attained in the presence of any mineral deficiency or imbalance. Vitamin D is not an essential nutrient for the intact rat, and in most mineral imbalances it exerts an unfavorable influence on body growth. While growth of parathyroidectomized rats fed diets with normal calcium and phosphorus content was, however, greatly improved by vitamin D, those fed an excess of calcium were stunted by this vitamin. Diets moderate to severely deficient in either calcium or phosphate produce rickets, but the degree of the disease is always comparatively greater with phosphate deficiency. Rickets were produced with about equal facility in parathyroidless and intact rats. Vitamin D ameliorated but did not preclude the appearance of rickets. Mineral deficiencies of even mild character impair skeletal mineralization as revealed by roentgenographic examination and bone ash determinations. Deposition of mineral in the kidneys and urinary tract occurred only with diets having more than normal content of calcium or phosphate. High calcium diets produced stones in the urinary tract resulting in hydronephrosis. High phosphate diets produced nephrocalcinosis only.

130. Functional Maxillary Orthopedics as Aid in Prosthetics. *Richard Grohs, and Leopold Petrik, Vienna, Austria.* In cases of distal occlusion or displacement after loss of teeth a functionally and esthetically successful prosthetic treatment is possible only after orthodontic preparation. Only thus can a loosening of the tissues and a functional preparedness be achieved, without which the desirable occlusion cannot be permanent. Functional maxillary orthopedics (Andresen) is the method of choice. After several months of treatment with an activator, the mandible can be brought into centric occlusion. Experiments are under way to treat edentulous patients in the same manner.

131. Geographic Variations of Dental Caries in Oregon. V. Dental Caries Among School Children in the Willamette Valley Region. *Demetrios M. Hadjimarkos, and Clara A. Storvick, Oregon State College, Corvallis, Ore.* Continuing our epidemiological studies on caries in various geographic regions of Oregon, we conducted an investigation on the prevalence of caries and some of the ecological factors which may have an influence on it in the counties of Marion and Benton of the Willamette Valley region of the state. The subjects of the present investigation were native born and reared white school children 14, 15, and 16 years of age who were drawn from all the schools of both counties. Altogether, 593 children were examined in the 2 counties. The procedure and criteria for the examinations were the same as in our previous studies (*J. D. Res.*, 28: 415, 1949). All examinations were made by the senior author. The caries experience of the subjects in the 3 age groups combined was found to be 11.8 D. M. F. teeth for Marion, and 13.1 D. M. F. teeth for Benton. The environmental factors studied were the following: (1) fluoride content of all the public water supplies in both counties, together with a retroactive investigation of these water supplies, (2) ratio of dentists to population, (3) consumption of candy bars and carbonated beverages, and (4) climatological data and altitude. The results of the present study, together with those of our previous investigations, indicate that an inverse relationship between dental caries and amount of sunshine in combination with altitude is the outstanding factor associated with differences observed in caries prevalence in the 3 geographic regions of Oregon investigated so far.

132. Some Observations on the Oral Cavity of the Syrian Hamster and the Lack of Effect of Pouchectomy on Experimental Dental Caries. *John W. Hein, James K. Avery, and Robert W. Clubb, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* The pH of dietary debris in the pouches of 37 adult Syrian hamsters 94 days on Keyes' caries-producing diet averaged 6.2 (5.3 to 7). The pH of saliva in the region of the sublingual and submaxillary ducts beneath the tongue averaged 9.5 (8 to 10). The orifices of the parotid ducts were found behind a tab of tissue located anterior and lateral to the maxillary first molars by the superior border of the opening into the pouch. The pH in this region averaged 7.9 (7 to 9). Semiquantitative analyses for amylase activity of homogenates of salivary glands of hamsters and albino rats gave for the range of sensitivity employed: parotids, same activity; submaxillary—hamster, none—rat, one half of parotid; sublingual, none. The presence of food, low pH, lactobacilli, and the possibility of salivary amylase activity in the pouch suggested the testing of the effect of unilateral and bilateral pouchectomy on experimental dental caries. The pouches were easily removed intraorally by inverting the pouch with tweezers, tying off proximally, and severing. Fifty-seven hamsters were weaned and separated by sex and littermate distribution into 3 groups: Group I was the control; in Group II the left pouches were removed; in Group III both pouches were removed. Keyes'

caries-producing diet was given for 94 days when the animals were sacrificed and the caries scored by Keyes' method. Removal of the pouch caused no statistically significant differences in caries activity confirming the report of Granados (*J. D. Res.*, 1949). Caries scores differed between sexes within and between all groups, the mean total score for males being 101, for females, 56.

133. Further Studies on the Inhibition of Experimental Caries by Sodium Copper Chlorophyllin. *John W. Hein and William G. Shafer, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y. and School of Dentistry, Indiana University, Indianapolis, Ind.* Previous studies by the authors have shown sodium copper chlorophyllin of approximately 75 per cent purity to be effective in (a) inhibiting or decreasing in vitro acid production in saliva-carbohydrate mixtures, and (b) decreasing the extent of experimental caries in the Syrian hamster. Recently it has been possible to obtain chlorophyll with a stated purity of 93.2 per cent. In a series of in vitro experiments it was found that whereas a 1:2,000 dilution of 75 per cent chlorophyll would inhibit acid production such that a pH of 6.0 was observed after 5 hours of saliva-carbohydrate incubation, a 1:10,000 dilution of 93 per cent chlorophyll was just as effective. Using a level of 1:500 under similar conditions, it was found that a pH of 8.0 could be maintained for at least 96 hours with 93 per cent chlorophyll but only for 24 hours with the 75 per cent grade. On the basis of these findings, it seemed advisable to retest the 93 per cent compound for experimental caries inhibition in the hamster. Fifteen males and 13 females were divided by sex and littermates into control and experimental groups. At 30 days of age the animals were given Keyes' caries-producing diet. The controls received distilled water ad libitum, the experimentals a 1:500 solution of 93.2 per cent chlorophyll ad libitum. After 110 days the animals were sacrificed and carious lesions scored according to the method of Keyes. The mean caries scores were found to be: control males, 58; experimental males (receiving chlorophyll), 20; control females, 27; experimental females, 8.

134. A Study of Some of the Physiological Effects From Local Anesthetic Drugs in Human Subjects. *Mellor R. Holland, School of Dentistry, University of Minnesota, Minneapolis, Minn.* The experiment was designed to compare some systemic reactions following the intra-oral injections of 2 and 4 per cent procaine with and without 1:50,000 epinephrine, with those reactions occurring after injection of Ringer's solution. Eighteen subjects received a 4 c.c. injection of each solution on separate days independent of oral surgical or operative procedures. Prior to each test, the subjects rested for 5 minutes. Room temperature, relative humidity, oral temperature, blood pressure and electrocardiographic recordings were made. During injection and for 10 minutes thereafter repeated electrocardiographic and blood pressure recordings were made and objective symptoms of reaction were noted. After the test, the subject was asked to relate any experience of subjective symptoms of reaction. The important results were as follows: (1) None of the subjects had untoward symptoms with Ringer's solution, but several experienced undesirable side effects with the anesthetic drugs suggesting the toxic property of these solutions. Procaine itself occasionally produced warm sensations, perspiration of the hands and forehead, and dizziness. The procaine-epinephrine solutions often created similar but more pronounced symptoms plus the development of tremors and strong heart palpitations. (2) The electrocardiograms showed that Ringer's solution and procaine did not create any significant changes in the cardiac pattern. With the procaine-epinephrine solutions, there were 6 cases in which

the T wave was definitely depressed indicating myocardial anoxia. (3) These results suggest that many of the annoying side effects following local anesthetic injection may be on a toxic rather than psychic basis. In addition, the results indicated that epinephrine is more frequently the causative agent of these toxic reactions.

135. Radiocalcium Uptake as Compared With Alizarin in the Mineralization of Bone. *Joseph R. Jarabak, Northwestern University Dental School, Chicago, Ill.* Six rats aged 31, 192 and 306 days were divided into two groups. One rat at each age level was given a 2 per cent solution and the other rat was given a 4 per cent solution of alizarin red S, interperitoneally. The dosage was computed on the basis of 100 milligrams per kilogram of body weight for the 2 per cent solution and 200 milligrams per kilogram of body weight for the 4 per cent solution. Two hours later the rats were given 40 microcuries of radiocalcium (^{45}Ca) per 100 grams of body weight, interperitoneally. The animals were sacrificed by decapitation 3 hours later, and the heads were boiled in 10 per cent solution of sodium hydroxide until the skulls were free of tissue. The skull of the 31-day-old rat showed deep staining with the 2 per cent solution and very diffuse staining with the 4 per cent solution of alizarin red. The skull of the 192-day-old rat which was given the 2 per cent solution showed very faint staining along the superior lateral margin of the zygomatic arch, in the sutures and along the alveolar margins. In the mandible a similar staining appeared along the inferior border, the alveolar process and the surface of the condyle. The 4 per cent solution gave good selective staining in the above areas. In the skulls of the 306-day-old rats, there was no visible staining with the 2 per cent solution and faint staining with the 4 per cent solution. Sagittal and cross sections of the skulls and mandible indicate that there is an uptake of calcium in all of the areas indicated by the alizarin. Although there was no staining with the 2 per cent solution of alizarin in the skull of the 306-day-old rat, the calcium uptake was in the areas previously described for the alizarin. Although alizarin has been recognized as an indicator of bone mineralization, its sensitivity is not comparable with radiocalcium (^{45}Ca).

136. The Uptake of Radiocalcium (^{45}Ca) in the Skull and Face of the Rat. *Joseph R. Jarabak and Maclyn Kamins, Northwestern University Dental School, Chicago, Ill.* Radioautographs were made of sagittal sections taken through the skull of 2 rats. These rats were given 40 microcuries radiocalcium (^{45}Ca) per 100 grams of body weight, interperitoneally, 3 hours before they were sacrificed. The sagittal section taken through the root area of the upper central incisor and molar area shows extensive deposition in the formative zone of the incisor. There is also an area of deposition along the alveolus of the incisor. The uptake in the upper molar area is principally in the dentin with a very light uptake along the dento-enamel junction. There is an extensive area of calcium uptake along the upper alveolar ridge and extending upward into the maxilla. A section taken anteriorly, through the malar portion of the zygomatic arch and, posteriorly, through the tympanic bulla shows areas of the greatest uptake of calcium along the lateral superior aspect of the zygomatic arch. The inferior border of the zygomatic arch is also an area of heavy uptake. The inferior aspect of the tympanic bulla, the coronal and frontal nasal sutures as well as the inner cranial table, show extensive deposition of radiocalcium (^{45}Ca). The presphenoid and basisphenoid show very small uptake in their respective bodies but considerable uptake in the region of the spheno occipital and basispheno-presphenoidal synchondrosies.

137. Alizarin as an Indicator of Bone Growth. *Joseph R. Jarabak and LeRoy Vehe, Northwestern University Dental School, Chicago, Ill.* Twenty five rats were divided in 5 groups of 5 rats each. The first group were 30 days old; the second, 60; the third, 120; the fourth, 180; and the last, 260 days old. All of the groups were given, interperitoneally, a single dose of a 2 per cent isotonic solution of alizarin red on the basis of 100 mg. per kilogram of body weight. The rats were sacrificed by decapitation on the next day, and the skulls were boiled in 10 per cent solution of NaOH until the tissue was removed. In the first group all of the skulls were very diffusely stained. The second group began to show selective staining in the mandible, maxilla, zygomatic arches and sutures. In the third group the staining localized in the same areas as in the second group; however, the stain was very faint. In the fourth group the staining was very light on the outer surface of the zygomatic arch in the cranial sutures, and along the inferior border of the mandible. The staining extended along the masseteric line to the distal border, from the mandibular angle and to the head of the condyle. In the last group there was no staining. Another group of 5 rats, 260 days old, were given a 4 per cent solution or 200 mg. per kilogram of body weight. The skulls indicate selective staining equal in density to that of the 60-day-old rat skulls in the first group. This experiment indicates that alizarin in sufficient amounts will stain bone in identical areas irrespective of age.

138. Is Caries Incidence Increasing? *Frances Krasnow, The Guggenheim Dental Clinic, New York, N. Y.* Consideration of the many factors entering a study which may involve changing caries rates indicated that findings at 3 years of age would have the fewest variables. These children generally receive the best health care commensurate with existing knowledge and are less apt to be exposed to irregular socio-economic situations. Any difference in the dental picture for the 2 periods selected, the first 1931 to 1933 and the second 1944 to 1947 may then be construed as due to changing concepts of the requirements for optimal physiological well being. The data reported are based on First Completion records. A summary comparison of the 2 sets of figures is herein tabulated. For 1944 to 1947, there is manifested a drop of 38.9 per cent or 2.1 decayed, missing,

PERIOD	CASES	CARIES FREE	CARIES PER CASE	CARIES PER CARIOUS CASE	D. M. F.
1931-33	100	24.0%	5.4	7.1	Same
1944-47	161	41.6%	3.3	5.6	Same
Relative difference		66.7% more in 1944-47	21.1% less in 1944-47	38.9% less in 1944-47	

or filled teeth per patient. If calculations are made on the basis of carious cases only, the average D.M.F. falls from 7.1 to 5.6. Apparently then, the caries attack of susceptible children in 1944 to 1947 is 21.1 per cent lower than that of 1931 to 1933. Also, a rise of 66.7 per cent caries free is disclosed. These results are poignantly significant since the children of the 2 periods are equal in age as well as born and raised under similar circumstances regarding income status and environment. The conclusion seems warranted that the frequency of caries can be conditioned. Moreover, the high percentage caries free is objective evidence for the value of education and points to the need of accentuated concerted effort urging the adoption of preventive physiologic measures in steering toward better dental health.

139. A Reevaluation of the Mechanism of Pulp Response to Injury. *S. J. Kreshover, R. L. Simpson and R. M. Roberts, School of Dentistry, Medical*

College of Virginia, Richmond, Va. The healing response of experimentally exposed pulps was studied in 7 dogs and 10 human beings to demonstrate the relative importance of space maintenance over exposures and the use of $Ca(OH)_2$. Operative procedures were carried out on one group comprised of 44 dog teeth and 8 human teeth so as to expose their pulps. Cavities were sealed with paraffin and covered by cement. In the human teeth and 19 dog teeth, a space was maintained between the paraffin and pulp. In 6 of the latter teeth, the pulps were first treated with $Ca(OH)_2$. In the remaining 25 dog teeth, paraffin discs were adapted directly onto the pulps, $Ca(OH)_2$ being employed in 10 instances. In a second group, 7 anterior teeth of dogs and 3 incisors of human beings were fractured through their coronal portions and paraffin lined metal crowns placed over them so as to maintain a space over the exposed pulps. Two other canine dog teeth were similarly prepared but received an initial application of $Ca(OH)_2$. Time intervals from operation date to tooth sacrifice ranged from 14 to 91 days. Histologic findings in Group I ranged from complete pulp degeneration and necrosis to osteo-dentin formation and secondary dentin bridging below the exposure sites. In most instances, calcific repair was present in those teeth treated without pressure and there was little evidence that preliminary use of $Ca(OH)_2$ made for significant differences in the resultant picture. On the other hand, in those teeth treated without space maintenance, there was marked degenerative change when $Ca(OH)_2$ was not used but definite repair when it was used. Findings in Group II were primarily those of granulation tissue formation with little evidence of calcific repair regardless of the use of $Ca(OH)_2$. Additional cases are still under study.

140. Photoelectric Determination of Carious Areas From Mouth Charts. *V. F. Lisanti, A. Yurkstas, W. Curby, Tufts College Dental School, Boston, Mass.* An instrument has been constructed to determine irregular areas such as found on mouth charts of carious lesions. The mouth charts are printed on an offset press on standard bond paper. The instrument employs a photosensitive plate which records the amount of light transmitted through the charts. Area readings can be taken at the rate of 200 charts per hour.

141. Delinquency and Coronal Fractures of Anterior Teeth. *Martin Marcus, New Jersey State Diagnostic Center, Menlo Park, N. J.* In the course of regular medical and dental examinations of boys and girls in the age range between 8 and 17 years, a high frequency of fractured anterior teeth was observed. Since the finding was corroborated as the series of cases accumulated, a detailed evaluation was undertaken. The figures tabulated revealed the pertinent differences between delinquents and a corresponding age group of non-

	DELINQUENTS			NON-DELINQUENTS		
	CASES	NUMBER FRACTURED	PERCENTAGE	CASES	FRACTURED	PERCENTAGE
Boys	71	35	49.2	100	18	18
Girls	29	11	37.9	50	7	14
Total	100	46	46	150	25	16

delinquent public school children. Of 71 delinquent boys, 35 (49.3 per cent) presented coronal fractures of anterior teeth; for the girls in this series, there were only 37.9 per cent (11 of 29) with such lesions. Considering these cases together, 46 of the 100 disclosed the lesion. In contrast, the nondelinquent 150 children used as the control, included only 25 (16 per cent) who had coronal fractures of the incisors. Subdividing the sexes, 18 of 100 boys, and 7 of 50

girls showed the involvements. From statistical calculation, it became evident that the incidence of coronal fractures in the delinquent group is definitely greater than that of the nondelinquent series. Plans are being formulated to determine possibly the metabolic basis for these findings.

142. Effect of Topical Sodium Fluoride on Dental Caries During Adolescence. *Cyril D. Marshall-Day, Tufts College Dental School, Boston, Mass., and Eastman Dental Dispensary, Rochester, N. Y.* Clinical and x-ray oral examinations were made of Rochester High School girls aged 14 to 15 years, 3 months, 100 being available for re-examination 14 months later. The teeth of right or left quadrants were isolated, dried, and treated with 2 per cent sodium fluoride 4 times within a maximum of 8 days, ensuring passage of Na F to interproximal surfaces by Na F-saturated, unwaxed, floss silk. As an added safeguard against contamination by NaF from the treated side, opposite quadrants were first coated with neutral varnish. First and final examinations were recorded on separate charts and all examinations made by the same examiner, unaware of the identity of treated quadrants. The necessity for x-ray examinations in such investigations is emphasized by the large number (9.2 per subject), and wide variation in the number of cavities disclosed or requiring x-ray confirmation in the treated and control quadrants respectively, e.g. 11:3, 0:7, 12:4, 10:18. The total of 923 x-ray cavities at the first examination was considerably greater than the total of 730 new D.F. surfaces which developed during the experimental period. Of the 100 cases, 54 showed either a greater or the same numerical increment in carious surfaces in the NaF-treated quadrants compared with control quadrants. The number of new D.F. teeth per cent was 13.5 and 15.2 in the NaF-treated and control quadrants respectively (difference 1.7 per cent). Of 4,539 NaF-treated sound surfaces, 346 (7.6 per cent) became carious, and 384 (8.4 per cent) of 4,596 in the control quadrants, a difference of 0.8 surface per cent. Calculated on the basis of the average number of sound teeth and surfaces present in this age group, the caries reduction under these conditions is only 1 tooth in every 5 children, or 1 surface in every 3 children. Statistically the differences were not significant. The possibility that the use of neutral varnish on the control quadrants may have affected the result cannot be entirely discarded; but the high caries increment in quadrants treated with NaF, despite every effort to enhance the action of the drug, does not demonstrate a high degree of effectiveness at this age.

143. Action of Amalgam on Dentin. *Maury Massler, and Thomas K. Barber, College of Dentistry, University of Illinois, Chicago, Ill.* Greenish-black discoloration of the dentin was found in 85 per cent of 300 freshly extracted permanent teeth with amalgam fillings. The discolored dentin was differentiated from carious dentin by its color, reaction to peroxide, radiopacity and preservation of tubular structure. Spectographic analysis of the discolored dentin revealed the presence of small amounts of all the metals present in amalgam and relatively large amounts of mercury (0.5-5.0 per cent). Galvanic action within a single amalgam filling has been demonstrated by Schoonover and Souder (1941). The metallic particles are probably diffused through the dentin as a result of such galvanic action and deposited as metallic sulfides. This hypothesis was tested by reproducing the discoloration in amalgam tooth models using an external source of electric current. Cavities were prepared in freshly extracted teeth and filled with amalgam. A current was then passed through the amalgam to an electrode in the pulp. Metallic diffusion through and discoloration of the dentin could be demonstrated in a few minutes by precipitation as metallic sulfides. A cement base prevented the diffusion of metals through the dentin.

144. Action of Acid on Topically Fluoridized Intact Enamel Surfaces. *Maury Massler, Carlos Tapia (Depassier), and Elizabeth Zwemer, College of Dentistry, University of Illinois, Chicago, Ill.* Freshly extracted permanent teeth were immersed in sodium fluoride solutions so that only the mesial or distal half of the enamel was covered by the solution for varying periods of time. The tooth was then totally immersed in lactic acid buffered to pH 3.9 and observed under a binocular microscope (×10 to ×60). Increasing concentrations of NaF solutions (from 0.1 per cent to 4 per cent) resulted in longer times required to etch the enamel. The relationship followed a parabolic curve. Longer immersion time in fluoride solution (from 5 minutes to twelve hours) resulted in longer times required to etch the enamel. The relationship was linear. It was estimated that the fluoride ions did not penetrate more than 30 microns below the surface of the enamel since acid action was not inhibited below that depth. Not all teeth could be successfully fluoridized. In our series only 30 per cent of all teeth accepted the fluoride ions. Age of the tooth was not a factor since teeth from old persons were affected in the same way as teeth from younger persons. The most important single factor determining successful fluoridization of the enamel was careful cleansing of the enamel surface prior to immersion in fluoride solution.

145. A New Method for the Diagnosis of a Focal Infection. *Hermann Mathis, Graz, Austria.* It has been shown that the determination of capillary resistance offers a simple and reliable method for the diagnosis of foci of infection. The simple apparatus consists of a 10 liter bottle with a rubber stopper through which one tube leads to a water aspirator, another to a mercury manometer and a glass suction head of 22 mm. diameter. Under a pressure of 200 mm. mercury the suction head is placed on the skin of the neck between the trapezius and sternocleidomastoid muscles and held there for one minute. The petechiae inside a circle of 12 mm. diameter are counted, with the aid of a magnifying glass. The presence of more than 14 petechiae is characteristic for the presence of focal infection. The method is sensitive and reliable, especially for the control of therapeutic measures.

146. The Role of Vitamin B_{12} in the Treatment of Glossopyrosis. A Preliminary Report. *Samuel C. Miller, Norman E. Alderman, and Sheldon Ross, School of Dentistry, New York University, New York, N. Y.* Various types of treatment have been advocated for the symptoms of "burning tongue" or glossopyrosis. Vitamin B_{12} was selected for study to determine its value in oral medicine, because it alleviates the symptoms of glossitis associated with the megaloblastic anemias. Six women, ranging from 33 to 60 years of age (4 of whom were past the climacteric) were treated for glossopyrosis with Vitamin B_{12} orally and intramuscularly. The duration of their symptoms previous to treatment ranged from 1 year to over 10 years; 4 presented some degree of cancerophobia. All 6 patients gave emotional histories ranging from mild to severe. Complete blood counts were performed on all patients prior to therapy. The results were within normal limits except for elevated lymphocytic counts in all cases. Each patient received 15 micrograms of crystalline vitamin B_{12} intramuscularly weekly for a period of 1 to 2 months. The oral dosage of vitamin B_{12} varied from 5 micrograms daily to no oral dosage, depending on the severity of the condition. After 3 months of treatment, all 6 patients displayed a decreased incidence of subjective symptoms. Objectively, a reduction of the glossitis and a return toward normalcy of the tongue was noted. The therapy was discontinued on 1 patient after alleviation of all symptoms, and the patient has been asymptomatic for 8 months. The remaining 5 patients are continuing

oral dosage as necessary. Further experimentation with vitamin B_{12}, using a control group, will enable us to determine the complete value of this substance in the treatment of glossopyrosis.

147. Practical Prescription Procedures in Vitamin Therapy. A Preliminary Report. *Samuel Charles Miller, Harry Roth, and Marvin Simring, New York University College of Dentistry, New York, N. Y.* The effectiveness of various vitamin preparations on symptoms commonly associated with vitamin deficiencies was studied on a group of 40 periodontal patients. A special diagnostic history chart was devised in order to standardize the data. Analysis of the data showed that following vitamin therapy there was most marked improvement in gingival bleeding and neuropsychiatric symptoms. Gingival bleeding was reported by 24 of the 40 patients and 14 of the 24 improved under vitamin therapy. Mild psychological malaise and a tendency to become fatigued easily were exhibited by 17 of the 40 patients but 16 of the 17 improved under vitamin therapy. Withdrawal of vitamin therapy in 6 cases caused a recurrence of the original symptoms. The combination of therapeutic dosage of a multiple vitamin supplement with a desiccated liver adjunct was found to be most effective in achieving these results. It is suggested that a similar nutritional etiologic factor may be operative in both gingival bleeding and psychological malaise since both symptoms were present, and both yielded strikingly to the same nutritional therapy.

148. Ammoniated Dentifrice and Periodontal Disease in the Hamster. *David F. Mitchell, and D. S. Chernausek, University of Minnesota School of Dentistry, Minneapolis, Minn.* Previous reports have dealt with periodontal disease in the hamster maintained on a soft, high carbohydrate, cariogenic diet. Apparently the loss of supporting structures of the molars is associated with accumulations of soft masses of food and debris similar to materia alba. The scoring method, previously reported, is based on the assignment of the unit 1 to the involvement of the gingival crevice of any surface (except proximal). This report is collateral with previously reported studies of the effect of ammoniated dentifrices on dental caries in the hamster. The mean periodontal scores of the 4 major groups follow: I, Control, 6.8; II, Toothbrushing, control dentifrice, 7.6; III, Brushing, experimental dentifrice, 3.7; IV, Ingestion, experimental dentifrice, 5.1; (I & II, 7.2; III & IV, 4.5). Results seem to indicate that brushing, per se (once per day) does not influence the periodontal score (contrary to results observed in dental caries). However, the groups receiving ammoniated compounds (III & IV) apparently show a reduction in periodontal disease.

149. Histological Findings in the Salivary Glands of the Rat Following Sodium Fluoride Administration. *Alfred L. Ogilvie, University of California College of Dentistry, San Francisco, Calif.* Although the salivary glands are known to be a pathway of fluoride excretion there are no references to specific fluoride-produced change in these structures. An experiment was conducted involving the intraperitoneal administration of sodium fluoride to 20 rats, and the subsequent study of their parotid, submaxillary, and sublingual glands. Definite modifications were observed in the alveolar cells of both parotid and submaxillary glands. In the submaxillary alveolar cells there were changes in the proportionate number of one type of cell to another, enlargement of nuclei, staining irregularity, and vacuolation of the cytoplasm. The same cytoplasmic and nuclear changes, as well as an increased mitotic division, were found in the alveolar cells of the parotid gland. The sublingual gland remained unaltered.

It is evident that serous cells of both parotid and submaxillary glands are specifically affected by the fluoride ion.

150. Traumatic Bone Cysts. *Eli Olech, Harry Sicher, and Joseph P. Weinmann, Departments of Oral and Maxillofacial Surgery and Oral Pathology, University of Illinois College of Dentistry, and Department of Anatomy, Loyola University Dental School, Chicago, Ill.* Traumatic bone cysts are usually diagnosed incidental to routine roentgenographic examination. They are characterized by a history of trauma but are asymptomatic with a slow, painless increase in size without bulging of the involved bone. Traumatic bone cysts develop either in the metaphyses of long bones or in the mandible in growing individuals. Eight traumatic cysts of the mandible are the basis of this study. It is interesting that, while the cysts encroach upon the root ends of the contiguous teeth, their pulps remain vital. Traumatic cysts seem to be restricted to parts of the skeleton where spongy bone contains hemopoietic marrow and is enclosed in a strong compacta. The rich blood supply of red marrow and the fragility of the walls of the medullary sinusoids explains the possible great extent of the primary hematoma. Though organization and spontaneous healing occur frequently, in some instances this process is inhibited if the damaged bone trabeculae face an inactive blood clot and are removed by the slow process of undermining and tunnelling resorption. In this case, before active connective tissue gains contact with the clot, the clot liquifies. Disturbances of venous drainage in the neighborhood leads to serous inflammation (phlegmasia) of the bone marrow and to a slow expansion of the cyst. Surgical exposure reveals a cavity, which may be either empty or filled with a serous or hemorrhagic fluid, and which has no lining membrane visible clinically. Histologically, an epithelial lining is never present, and in most instances even a connective tissue lining is absent. Filling of the cystic cavity with a fresh blood clot that is in primary contact with the connective tissue of the flap leads to speedy healing.

151. A Radiographic Study of Variations in the Physiological Rest Position of the Mandible in Seventy Edentulous Individuals. *Edmund S. Olsen, School of Dentistry, University of Minnesota, Minneapolis, Minn.* This cephalometric radiographic study was undertaken to determine the effect of the wearing of artificial dentures on the stability of the physiological rest position of the mandible. Direct film measurements of face height from successive radiographs of rest position of the same individual without dentures in the mouth varied from 0.3 millimeter to 5.7 millimeters in 98.6 per cent of the cases. Like measurements of the same individuals with dentures in place varied from 0.1 millimeter to 10 millimeters. Therefore, it is suggested that the resting position of the edentulous mandible is not rigidly stable either with or without dentures in the mouth, but shows only a tendency toward stability in most cases. In comparing the average recorded measurement of rest position of an individual with dentures in the mouth with that of the same individual without dentures, the difference is from 0.2 millimeter to 7.2 millimeters in 97.1 per cent of the cases. The average rest measurement with dentures in place was greater than the comparable measurement without dentures in 82.9 per cent of the cases. From these findings it appears that the wearing of artificial dentures influences the resting face height of the individual when dentures are in place in the mouth. This observation should be carefully considered when rest position registrations are used clinically to establish vertical interarch relationships in complete denture practice.

152. Frequency of Occurrence of Actinomycetes and Other Pleomorphic Organisms in Carious Lesions of Human Teeth. *Masao Onisi, and*

James Nuckolls, University of California, College of Dentistry, San Francisco, Calif. Bacteriologic studies were made of 35 carious lesions. These included 17 early lesions involving only the enamel, 16 moderately advanced lesions involving both enamel and dentin, and 2 lesions which had advanced to pulp exposure. Employing liver extract agar (pH 7.1), tomato juice agar (pH 6.1), and malt agar (pH 5), a total of 166 strains were isolated from these lesions. Classified on the bases of morphologic and colonial characters, 133 of them were believed to be Actinomycetales. These were arbitrarily divided into 6 types according to their predominant morphologic character. Type III and Type V were associated with only the early lesion. Type I and Type II were most frequently associated with both the early and moderately advanced lesions, while Type IV and VI were less frequently associated with them. The lesions advanced to pulp exposure contained Type VI and, in addition, a variety of microorganisms other than Actinomycetales. These cultural observations were confirmed with microscopic examinations of direct smears made simultaneously from the same areas. The results indicate that certain members of the Actinomycetales are intimately related to the initiation and advancement of dental caries. Members of the genus Lactobacillus were infrequently found associated with the carious lesion in these studies. However, because of present difficulties in systematic classification of both Actinomycetales and Lactobacilli, particularly on a morphologic basis, the strains isolated are being studied further with respect to their lactic acid fermentation.

153. The Dual Functions, Motor and Secretory, in the Stomach of a Fish. *T. L. Patterson, Departments of Physiology, Hopkins Marine Station, Stanford University, Pacific Grove, Calif., Wayne University College of Medicine, and University of Detroit School of Dentistry, Detroit, Mich.* This contribution represents one of a long series of phylogenetic studies on comparative physiology of the gastric motor mechanism of vertebrate and invertebrate animals. (Patterson: *Ann. New York Acad. Sc.* **34**: 55, 1933.) A sedentary, marine fish, the bullhead or cabezone (*Scorpaenichthys marmoratus*) was selected as a suitable experimental type. Following anaesthetization, each fish was stomostomized and placed separately in an aquarium containing fresh, aerated, running, sea water. At first, gastric motility recordings (balloon-bromoform-manometer method) were made of the digestive and hunger movements, in which both exhibited continuous, rhythmical contractions, the strength of the contractions being somewhat greater during digestion than during hunger peristalsis, the latter ranging from 50 to 70 mm. of bromoform manometric pressure. The active contractions were completed in 20 seconds and were separated by periods of rest lasting 40 seconds. A modified periodicity existed. The method of recording permitted the fish to swim freely in the aquarium. Both digestive and hunger movements were inhibited when weak solutions of either alkali, or acid were introduced directly into the stomach and also by vigorous swimming. In the second phase of this investigation both the motor and secretory functions of the stomach were synchronously and graphically recorded, the gastric motility by the balloon and the gastric secretion by a rubber tube leading from the stomach to a drop recorder and receptacle for collection and testing of the gastric juice. This new technique makes possible the synchronous recording of gastric motility and gastric secretion, the secretion running parallel to the motility periods. Furthermore, the gastric juice may be collected and analyzed and the controlling nerve components investigated.

154. Effects of an Acute Febrile Disease, Poliomyelitis, on the Periodontium of Rhesus Monkeys. *Sigurd Ramfjord, School of Dentistry,*

University of Michigan, Ann Arbor, Mich. The repeatedly claimed relationship between acute systemic disease with high grade fever and periodontal impairment has not been previously investigated experimentally. Ninteen healthy Rhesus monkeys with gingival status ranging from normal to severe gingivitis were used for this experiment and 7 other healthy monkeys, exposed to similar environmental and dietary conditions were utilized as control animals. Color pictures and gingival biopsies were taken. The experimental animals were innoculated with poliomyelitis virus and observed daily. They developed a high grade fever lasting from 1 to 10 days and were sacrificed when they reached the moribund stage. Dental x-rays were taken and a necropsy performed immediately after death. Specimens for microscopic study were taken from jaws, teeth, temporomandibular joints, mesial end of tibia, and from visceral organs and lymph nodes. Hematoxylin and eosin stains were utilized for all of the tissues. In addition, Heidenhain's modification of Mallory's connective tissue stain (Azan) was used for sections of jaws and joints to investigate possible changes in the collagen fibers attributable to the acute febrile disease. The same procedure of examination was utilized for the control animals. Different stages of traumatism were observed in jaw sections. The histopathological picture of these lesions appeared indistinguishable from descriptions in the literature and photomicrographs of diffuse atrophy or periodontosis. There was no difference in susceptibility to these lesions comparing experimental animals and control animals. Changes attributable to poliomyelitis were not observed in the periodontium. Special connective tissue stains did not reveal any structural changes in joints or periodontal tissues. An association was noted between accumulation of debris on the teeth and gingivitis. Gingivitis was observed more frequently and found to be more severe around deciduous than permanent teeth. An inverse relationship was observed between the length of the root of deciduous teeth and the degree of gingival inflammation.

155. The Effect of a Topical Application of a 2 Per Cent Solution of NaF on the Oral Flora of Young Adults. *N. H. Rickles, Division of Dental Medicine, College of Dentistry, University of California, San Francisco, Calif.* An experimental group of 97 patients had their saliva samples incubated with a sucrose-indicator mixture, with the drop in pH of each sample being recorded each hour for 7 hours and the total acid produced at the end of the 7 hours measured in terms of c.c.'s of 0.02 N NaOH. A control group of 46 individuals similarly had their salivas evaluated for acidogenic properties. The experimental group then had 4 weekly applications of an aqueous 2 per cent solution of NaF to all their teeth, while the control group had 4 weekly applications of distilled water. One week after the final application all salivas of both experimental and control groups were again examined for the acidogenic properties of the oral flora. The differences in pH between the original and final samples at each hour and total acid at the end of 7 hours in each case was noted. On inspection it appeared that the experimental group's saliva had, on the average, produced less acid than had the control group. On statistical evaluation, these differences between the amounts of acid produced by the experimental and control groups after application of the 2 per cent NaF and distilled water, respectively, to the teeth were found not to be significant. The lactobacillus counts of the experimental and control groups were recorded before and after the application to the teeth of the 2 per cent NaF and distilled water, respectively. No significant difference existed between the mean increase of lactobacilli in the experimental and control groups.

156. The Effects of a Neutral and an Acid Solution of NaF on the Incidence of Dental Caries in Young Adults. *N. H. Rickles, Division of*

Dental Medicine, College of Dentistry, University of California, San Francisco, Calif. The effectiveness of two 2 per cent NaF solutions, with sodium lauryl sulfate incorporated as a wetting agent, was tested for their abilities to reduce the incidence of dental caries in young adults. The solutions tested were identical in all respects, except that one was at a pH of 7, while the other was at a pH of 3.5. Two groups of dental students of 25 individuals each were employed as test subjects. In each group the teeth on one side of the mouth were treated with a series of 4 applications of the indicated solution, with the teeth on the other side serving as controls. The group using the solution at a pH of 7 developed 48 new carious surfaces in the treated teeth after a 2 year observation period, where there had been 264 open lesions at the outset of the experiment. Eighty new lesions developed in the untreated teeth where 266 open lesions had been in existence at the beginning of the experiment. The difference in the two percentages—$^{48}/_{264}$ or 18 per cent and $^{80}/_{266}$ or 30 per cent —was highly significant. This may be interpreted as meaning that the 2 per cent NaF solution at a pH of 7, with a wetting agent added, is capable of significantly reducing the total caries increment in young adults. Similarly significant results were obtained in the special cases of interproximal and secondary caries. Using the solution at a pH of 3.5, no reduction of caries increment occurred in any category.

157. Roentgenographic Studies of the Jaws in Sickle Cell Anemia. *Irwin B. Robinson and Bernard G. Sarnat, University of Illinois College of Dentistry, Department of Oral and Maxillofacial Surgery, Chicago, Ill.* A survey of the literature reveals a number of reports on the roentgenographic changes in the bones in sickle cell anemia. No reference, however, is made to either the mandible or maxillae. Twenty Negro patients (8 females and 12 males), ranging in age from 10 to 46 years with an established diagnosis of sickle cell anemia were studied. Intra-oral dental, lateral jaw and skull roentgenograms were taken of each patient. The dental roentgenograms in 17 of the 20 patients showed significant changes characterized by: (1) a markedly increased generalized radiolucency, (2) a decreased number with a resulting sharper definition of the bone trabeculae which were frequently arranged in a stepladder-like manner in the alveolar bone, and (3) large multi-sized and multi-shaped marrow spaces. The lateral jaw plates showed a general increased radiolucency throughout the medullary portion of the mandible without the detailed pattern of the dental roentgenograms. In the roentgenograms of the skull widened diploe and pointed trabecular striations radiating outward, perpendicular to the inner table, were demonstrated in only one instance. This preliminary work suggests that the earliest and sometimes possibly the only roentgenographic changes in the bones in sickle cell anemia can be observed in the jaws by means of intra-oral dental roentgenograms.

158. Effect of Surgical Removal of the Condyle on the Growing Mandible: An Experimental Study in the Macaca Rhesus. *Bernard G. Sarnat, and Milton B. Engel, University of Illinois College of Dentistry, Department of Oral and Maxillofacial Surgery and Department of Orthodontia, Chicago, Ill.* The effects of resection of the condyle on mandibular growth were studied in a group of 10 Rhesus monkeys (5 unoperated controls, 2 with a unilateral condylectomy, and 3 with a bilateral condylectomy). The animals were operated at about 8 months. The postoperative survival period ranged from 4 to 17 months. Serial cephalometric roentgenograms were taken preoperatively, immediately after surgical removal of the condyle, and at 6 month

intervals subsequently. Impressions were taken of the denture preoperatively and subsequently at 6 month intervals. The skulls were dissected, studied grossly and roentgenographically. Examination of the serial cephalometric roentgenograms revealed diminished density of the ramus and remodelling. Serial records showed that the ramus failed to grow in height after the condyle was resected. Where the operative interference was unilateral, a marked asymmetry of the mandible and the middle third of the face developed. Beginning evidence of antegonial notching and minor changes in the contour of the body of the mandible were seen in animals with a postoperative survival beyond one year. Examination of the gross specimens confirmed the roentgenographic findings. The zygomatic arch and orbit were consistently lower on the operated side. There was a bony outgrowth, particularly on the medial of the area resected, which was covered with cartilage and resembled a condyle. This was more prominent in the animals with a longer postoperative survival. The new joint surfaces were anterior to the glenoid fossa. In no instance was there an ankylosis.

159. Osteosclerosis of the Jaws. *Franz Schoenbauer, Vienna, Austria.* Circumscribed areas of osteosclerosis in the jaws are quite frequent. Roentgenograms show sclerotic zones around a focus of inflammation as a reaction of the osteogenetic cells. A case is reported that is interesting because of the pain caused by an extensive sclerosis of the maxilla. The patient had complained for about 20 years of pain in the region of the upper left first incisor, canine, and first premolar. Extractions and osteotomies were without success. A biopsy of the sclerotic area revealed an almost entirely compact bone. Blood vessels and nerves were crowded in canals and covered by a thin layer of connective tissue only. In one area a nerve was caught in the bone without interference of connective tissue. These findings seem to explain the neuralgiform pain. Foci of perivascular round cell infiltration point to inflammation as the cause of the osteosclerosis.

160. Experimental Increase in Rate of Eruption and Growth of Rat Incisor by Eliminating Attrition. *Isaac Schour and Herman Medak, School of Dentistry, University of Illinois, Chicago, Ill.* The effect of the bi-weekly cutting off of the clinical portion of the upper left incisor of the rat was studied in 17 animals for a period of 3 days to 24 weeks. The uncut right incisor was used as a control. Upon sacrifice, the heads were split in half and x-rayed. The upper incisors were decalcified, embedded in celloidin and studied in midsagittal histologic sections. The findings were: (1) The rate of eruption of the cut incisors doubled; (2) roentgenograms showed an increase in all the dimensions of the cut incisor, and in the width of the pulp, and a corresponding decrease in the thickness of the dentin walls; (3) histologic sections showed: *a*, thinning of dentin wall; *b*, persistence of tall ameloblasts to gingival level; *c*, reduction of periodontal membrane to half its normal width. Incisal stress and function regulate and depress the rate of eruption. Removal of incisal stress by cutting from incisor releases the eruption potential so that it is allowed full expression. The experimental doubling of the rate of eruption in the continuously growing incisor is correlated with the corresponding increase in size and the apparent increase in mitotic activity of the odontogenic epithelium and the basal pulpal cells. The appositional functional life span of the ameloblasts and odontoblasts and their daily rate of work appear to be unaffected.

161. On the Biological Effect of Modern Orthodontic Appliances. *A. Martin Schwarz, Vienna, Austria.* The action of functional maxillary ortho-

pedics (Andresen-Haeupl) was explained heretofore by the stimulation of increased mandibular movements during the night. It was possible to disprove this theory by fixing electric contacts on the appliances that registered every occlusion. The removable appliances seem to work by continuous forces as do other orthodontic appliances. The active force is the tetanic muscle contractions. Each muscle contraction consists in reality of 50-200 single twitches per second. These contractions result in vibratory forces transmitted by the appliance to the tissues as a stimulus for adaptive changes.

162. Transplantation of Developing Teeth in the Cat. *Harry H. Shapiro, Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York, N. Y.* Developing permanent canine teeth were removed from the mandibles of young cats. In each case these were replaced by a developing maxillary incisor tooth from the jaw of another animal. These experiments were performed in order to test the capacity of the developing tooth to substitute for the tooth of another animal. In a previous report, the progress of such intra-alveolar tooth bud transplants was recorded by roentgenologic examination. The present study reports the histological findings in the early stages of the re-establishment of the transplants, which indicate that healthy developing teeth, transplanted without trauma or infection become established and continue development. Injured transplants or those in which infection occurs in the host do not develop.

163. Abrasions of Tooth Surface by Nylon and Natural Bristle Toothbrushes. *E. W. Skinner and Grace K. Takata, Northwestern University Dental School, Chicago, Ill.* The increase in surface roughness of highly polished dentin and enamel surfaces of human teeth has been determined after brushing with 6 different commercial brands of nylon bristle toothbrushes and with 5 different brands of natural bristle toothbrushes, using distilled water as the brushing medium. The surface roughness determinations were made with a Brush Surface Analyzer. When the dentin surfaces were brushed by hand for 3 minutes, the surface was roughened slightly more by the nylon bristles than by the natural bristles. When the same surfaces were brushed by machine with 10,000 strokes, no significant difference in increase in surface roughness was produced by the two types of bristles. Regardless of the conditions of brushing, no measurable increase in surface roughness of enamel surfaces occurred at any time.

164. Mucinous Polysaccaride Production From Sucrose by Oral Bacteria. *Marshall L. Snyder, and Dorothy F. Donnelly, School of Dentistry, University of Oregon, Portland, Ore.* The production of mucinous colonies on sucrose-containing media by oral bacteria offers a convenient method for the isolation of organisms causing this reaction. As part of the study on a possible relationship between this synthesis and mucinous plaque formation, an analysis of the media employed was conducted to establish the influence of each component. Mucinous colonies were obtained in a medium composed by the following minima: 3 per cent sucrose, 1 per cent tryptose (Bacto), 0.5 per cent agar, and pH 6.0. Optimal results in respect to colony size were obtained with a medium composed as follows: 5 per cent sucrose, 2 per cent tryptose, 1.5 per cent agar, and pH 7.4. Phosphate in the monobasicpotassium form inhibited the development of mucinous colonies. Blood did not improve the medium. In more than 300 specimens of saliva from representative age groups mucinous colonies were constantly cultured in numbers ranging from 4.5×10^6 to 5.0×10^9

per ml. saliva. Two basic colony types with transitional stages were encountered: the first was a soft smooth colony easily emulsified while the other was rough, granular, of cut glass appearance and extremely difficult to emulsify. The rough type was more frequently observed in the older groups. These colonies were made up of gram-positive cocci in chains. Their identity was accepted as *Streptococcus salivarius*. No other organism in these specimens of saliva was observed to form colonies similar to those of the streptococci on sucrose agar under aerobic conditions irrespective of whether the medium contained blood or penicillin. There was no correlation between the incidence of these organisms or colony types and numbers of lactobacilli in respective specimens of saliva.

165. Disturbances of the Ear Caused by Changes of the Masticatory Apparatus. *K. L. Spring, Vienna, Austria.* In edentulous individuals with a loss of vertical dimension, an impairment of hearing acuity can sometimes be observed. The author denies a purely mechanical explanation by posterior displacement of the condyle and compression of the external acoustic meatus and the Eustachian tube. The cause of the loss of hearing acuity is to be found in a functional disturbance of the muscles of deglutition that are thrown off balance by the closing of the bite. Thus the normal opening of the tube, effected by the tensor palati muscle, and the aeration of the middle ear are inhibited. Pain in or around the ear, tinnitus, tenderness of the mandibular articulation, pain in the forehead, eyes, tongue, or pharynx (Costen's syndrome) are the consequence of arthritic changes of the articulation that are caused by abnormal stresses. Only cooperation of the physician and dentist can lead to successful treatment.

166. A Micro Antimony Electrode Suitable for Intraoral pH Measurements. *F. C. Thompson, and Finn Brudevold, Eastman Dental Dispensary, Rochester, N. Y.* A micro antimony electrode has been developed which is sealed within glass tubing of an outer diameter of about one millimeter. A piece of glass tubing containing antimony is heated until the metal liquefies, and since the glass becomes soft at the melting point of antimony, the tubing may be drawn to any desired dimension. The tube containing the solidified antimony may be reheated and given any convenient shape. A small portion of glass is ground away at the working end, and the protruding antimony is highly polished to a point. A silver wire, heated and embedded in the antimony at the other end of the electrode connects it with the shielded wire of the pH meter. The accuracy of the micro electrode is within 0.2 pH units in the range pH 4 to 7.5. The electrode may be employed in the determination of pH values in various lesions, interdental spaces, and periodontal pockets. With a capillary KCl bridge it is also suitable for studying the decalcification of minute areas of the enamel surface.

167. Effects of Selenium on the Incidence of Dental Caries in White Rats. *M. G. Wheatcroft, J. A. English, and C. A. Schlack, Naval Medical Research Institute, Bethesda, Md.* Eighty white rats, consisting of 40 males and 40 females, from a controlled colony, were maintained 100 days on a coarse corn cariogenic diet. The animals were placed in 4 groups of 20 rats each. Selenium (Na_2SeO_3) was administered intraperitonally to determine whether small doses would give a protective effect against caries. One group received 1 mg. per kilogram of body weight as a daily dose. A second group received 0.5 mg. per kilogram of body weight, and a third 0.2 mg. per kilogram. The fourth group was used as a control. Analysis of variance showed treatment to

cause no significant difference in caries incidence between groups at the 1 per cent level and a doubtful difference at the 5 per cent level. Trends were toward an increase of caries incidence in the group receiving the most selenium. The external appearance of most animals which received 0.5 and 1.0 mg. per kilogram of selenium indicated this dose was toxic. They had diarrhea, affected eyes and dull, matted fur. Histologically there was evidence of liver damage in over one-half of them and minor kidney damage in a third.

168. Pulp Reaction to Resin Filling Materials. *H. A. Zander, Tufts College Dental School, Boston, Mass.* Five selfpolymerizing resin filling materials were tested for their effect upon the dental pulp. Comparable cavities were prepared in dogs teeth and filled with the resin filling materials in addition to zinc oxide eugenol and silicates as controls. The results of this investigation are based on sections of 72 teeth from 6 dogs. All of the resin filling materials are irritating to the pulp but are less irritating than silicate cements and more than zinc oxide eugenol cements. There are variations in pulp response between the 5 resin-filling materials tested.

169. Complex Fluorides. Caries Reduction and Retention of Fluorine in the Bones and Teeth of the White Rat. *I. Zipkin, and F. J. McClure, National Institutes of Health, Bethesda, Md.* Rats on a cariogenic diet received equal quantities of 50 ppm F as NaF, Na_2SiF_6, Na_2PO_3F, KPF_6, and CF_3COONa in their respective drinking fluids. In addition, all but CF_3COONa were injected intraperitoneally so that litter mates received an amount of fluorine equal to that received orally. A control group received no fluoride. At the end of 90 days, control and fluoride rats were sacrificed, the teeth scored for caries, and the femurs and mandibles analyzed for fluorine. The incisors and the molar crowns were separated into dentin and enamel and analyzed for fluorine. Pronounced mottling was apparent in rats receiving NaF, Na_2SiF_6 and Na_2PO_3F orally or intraperitoneally, but no mottling was observed in any of the rats which received KPF_6 or CF_3COONa by either route. NaF, Na_2SiF_6 and Na_2PO_3F in the drinking fluid brought about a pronounced and similar reduction in caries whereas KPF_6 or CF_3COONa produced no caries inhibition. None of these fluorine compounds reduced rat caries when injected intraperitoneally. Rats receiving NaF, Na_2SiF_6 and Na_2PO_3F by either route absorbed significant and comparable quantities of fluorine in the bones and teeth, but rats receiving KPF_6 and CF_3COONa orally and by injection did not deposit fluorine in their bones and teeth. It seems apparent from these results that certain complex fluorides do not deposit fluorine in the skeletal and dental tissues, do not cause mottling, and also do not inhibit induced caries in rats.

XIII. Executive Proceedings and Register of Newly Elected Officers and Members

A. COUNCIL SESSIONS

Activities Between General Meetings

Between March, 1950 and March, 1951 the council determined the time and place for the current general session and nominated the council candidates for the 1951-1952 general officers for inclusion on the official ballot-forms.

First Session, Saturday, March 17, 1951

Council meeting was called to order by President Bibby at 1:30 P.M. with an attendance of 23. The secretary-treasurer submitted the following report covering the financial operations of the association for the 1950 calendar year.

Receipts

Dues, general fund share	$ 436.50	
Dues, *Journal of Dental Research* share	2,011.00	
C. V. Mosby Co. for Publication Committee	1,800.00	
Collections for Chicago Section	88.00	
Interest on bonds (endowment)	47.50	
Total		$4,383.00

Disbursements

On secretary-treasurer's budget		
Mailing	$ 98.19	
Clerical	114.18	
Printing	186.70	
Supplies, etc.	59.88	
Meeting	50.31	
Total		$ 509.26

Journal of Dental Research Budget

Expenses of Publication Committee	$ 418.00	
Publisher (C. V. Mosby Co.)	3,068.22	
Total		$3,486.22
Refunds, Chicago Section		84.00
Total disbursements		$4,079.48
Excess of receipts over disbursements		$ 303.52

On motion this report was approved and ordered for printing in the minutes of this meeting. A communication was read from Dr. Arthur H. Merritt announcing the incorporation of The William J. Gies Foundation for the Advancement of Dentistry as the successor of the William J. Gies Endowment Fund for the *Journal of Dental Research*. Of this fund, Dr. Merritt named himself as president and briefly stated its objectives. After some discussion this subject was referred to a committee of the trustees, Dr. T. J. Hill, chairman. A petition signed by M. K. Hine, J. L. T. Appleton, Harold J. Noyes, J. R. Blayney and Wilmer Souder called attention to the rapid increase of members professionally active whose dues were being remitted and requested a change in the by-laws to remedy this situation. They submitted a draft of a proposed amendment. After some discussion and modification this proposed by-law was approved for submission to the association at a coming executive session. On motion, the dues for the 1952 calendar year were set at $7.50 for North American members, at $6.00 per member for the new members who will be elected to membership at this meeting for the remainder of the 1951 calendar (March 19–December 31) year. A secretary-treasurer's budget of $675.00 for the 1951 calendar year was approved, divided as follows: Mailing, $100.00; clerical, $150.00; printing, $200.00; travel and meeting expense, $50.00; supplies and miscellaneous, $50.00, and unassigned, $125.00. On motion approved, the disbursement of $6.00 out of each $7.50 dues item collected to the C. V. Mosby Co. to cover the member block subscriptions to the *Journal of Dental Research* for the 1951 volume, and the disbursement of the $1,800.00 from the Mosby Co. for the expenses of and gratuities to the Publication Committee of the *Journal of Dental Research*. After some discussion Harold C. Hodge was authorized to investigate the advantages of this association joining the American Institute of Biological Sciences and report back to the council and association. Adjourned to Sunday, March 18, 1:30 P.M.

Second Session, March 18, 1951

Meeting called to order by President Bibby at 1:45 P.M. L. S. Fosdick reported that his committee had conferred with the Executive Committee of the American Association of Dental Schools with reference to the time and place of the 1952 meeting. Immediately after this conference the Executive Committee of the A. A. D. S. selected Colorado Springs and the Broadmoor Hotel as the place and March 24, 25, and 26 as the time for their 1952 session and offered this association the privilege of selecting the time and place for the 1953 meetings. After discussion it was moved and seconded that this association hold its 1952 meeting at the Broadmoor Hotel, Colorado Springs on March 21, 22, and 23; the vote was 12 in favor and 7 against. Because of the small majority in favor, final decision was postponed for submission to the whole council by a mail ballot. On motion the formation of a new section in Birmingham, Alabama was unanimously approved. A list of names of those individuals whose nominations to membership had been approved for election by the council committee appointed to examine the files was then read. (Committee: L. S. Fosdick, Paul Boyle and Harold J. Noyes). Three names were added to this list, Wm. B. Savchuck, Frederic R. Schiere and H. Henry Weisengreen, after examination of their credentials. The amended list, printed elsewhere in these proceedings, was then approved by a unanimous vote for submission to the association for election to membership at the final executive session. The tellers, D. Y. Burrill, Ned B. Williams and John Hein, reported that over 321 votes were cast but only that number counted as there was a small number of defective ballots. The election of the 1951-1952 officers as listed elsewhere in these proceedings was nearly unanimous. Report approved and tellers discharged with a vote of appreciation. On motion approved in principle the policy of the payment of honoraria to the editor and business manager of the *Journal of Dental Research* and the secretary-treasurer of the association, and authorized the formation of a committee to advise the council and association as to details. Motion authorized the appointment of a committee to select the time and place for the 1953 meeting. On motion adjourned to 8:45 A.M., Monday, March 19.

Third Session, March 19, 1951

Meeting called to order by President Fosdick at 8:45 A.M. The committee appointed to consider the subject of honoraria (D. Y. Burrill, F. A. Arnold, Jr., Paul C. Kitchin, and T. J. Hill) recommended that honoraria which are in no sense compensation for services rendered be voted to the secretary-treasurer of the Association and to the editor and business manager of the *Journal of Dental Research* in the amounts of $300.00, $1,000 and $500.00 respectively. On motion this report was approved and the disbursements authorized unanimously, on the basis of previous general approval by the council and association. Adjourned.

Councilors in attendance at one or more sessions: R. G. Agnew, F. A. Arnold, Jr., Gerrit Bevelander, B. G. Bibby, P. E. Boyle, A. W. Bryan, D. Y. Burrill, H. B. Clark, Jr., E. C. Dobbs, L. S. Fosdick, J. F. Hall, E. H. Hatton, J. W. Hein, T. J. Hill, H. J. Hodge, P. C. Kitchin, R. S. Manly, H. J. Myers, H. J. Noyes, F. J. Orland, F. C. Peyton, H. B. G. Robinson, N. S. Simmons, W. F. Swanson, W. L. Wylie. Total, 25.

B. REPORTS

REPORT OF THE BOARD OF EDITORS 1950-51

The request of the William J. Gies Foundation for the Advancement of Dentistry for suggestions of means by which it might assist the *Journal* was

discussed. The offer of a dentist to give a cash award for an essay on advantages of certain types of filling materials was discussed and the Board voted that it did not favor publishing notices of award offered by individuals.

The section known as the "Editor's Viewpoint" was discussed and it was unanimously voted that the section be continued. It was voted that the *Journal* publish a notice asking that surplus back issues be sent to the office of the Business Manager with transportation cost paid by that office.

The reports of the Editor, Business Manager, and Circulation Manager were approved.

Report of the Editor of the *Journal of Dental Research*

Volume 29 contained 866 pages (vs. 697 in Volume 28). This expansion was the result of a new contract with The C. V. Mosby Company to increase the size of the Journal annually from approximately 100 pages per issue to 150 pages per issue with a proportionate increase in subscription price. As a result 108 original papers (vs. 76 in Volume 28); 6 Editor's Viewpoints, 140 Abstracts from the General Meeting of the International Association of Dental Research, and four Section Meetings, the Executive Proceedings, the Index and Miscellaneous matter were published. As a result of the increase in pages, we are extremely happy to report much more prompt publication. For practical purposes we can state that all papers received before January 1, 1951 will be published in April, 1951 issue or an earlier issue. This is most encouraging and should increase the usefulness of the Journal and the desire to publish papers therein.

During the next few months the directory of members, compiled by Dr. H. C. Hodge, will be prepared for publication and made available in the Journal. In the past the huge backlog of scientific work made the feasibility of publication of this directory questionable.

The editor has had his duties increased by one-half through the proportionate increase in the size of the Journal. Many members have kindly served as consultants on special papers and have reduced this burden, somewhat.

Many of our foreign colleagues are availing themselves of the opportunity to publish in the Journal. Unfortunately, these papers usually present editorial difficulties but their continued contribution is encouraged. Papers from Canada, Denmark, Hungary, New Zealand, the Netherlands, Sweden, Argentina, China, and the Philippines appeared in Volume 29, or are in press.

Report of the Business Manager of the *Journal of Dental Research*

From Jan. 1, 1950 through Dec. 31, 1950

Balance on hand December 31, 1949		$1,196.78
Receipts:		
From back issues of the Journal	$1,251.51	
		$2,448.29
Disbursements:		
Clerical and miscellaneous	$426.91	
Storage and miscellaneous charges	170.68	
Postage	64.21	
Stationery	15.50	
	$ 677.30	
Balance		$1,770.99

REPORT OF THE CIRCULATION MANAGER

Journal of Dental Research—Period July-December, 1950

Six-month average circulation figures:

Individual subscribers:	1,754	
Membership-subscribers	532	
Total, Average Paid Circulation		2,286
Advertisers' copies	62	
Exchanges	113	
Samples	90	
		2,551

C. EXECUTIVE SESSIONS

First Session, 9:15 A.M., March 17, 1951

The meeting was called to order by President Bibby with a brief welcome to members and guests. The minutes of the twenty-eighth general meeting were then offered as printed in the *Journal of Dental Research* for October, 1950, pages 649-708. No corrections were made of this printed record and these minutes were declared approved. The balloting for general officers was announced in order and the polls opened by the naming of Dan Y. Burrill, Ned B. Williams and John W. Hein as tellers to receive and poll the ballots. Brief summaries of the financial reports were read and ordered to the Council for examination and recommendations. The resignations of E. C. McBeath, A. E. Treloar, and John B. Williams were reported. The executive session was then adjourned to Sunday evening.

Second Session, Sunday, 8:15 P.M., March 18, 1951

The meeting was called to order by President Bibby and the report of the council activities was made the first order of business and read by the secretary-treasurer. The following items were emphasized by individual and specific submission to the association for action. (1) Approval of the budgets and the disbursements for the 1951 and 1952 years as printed in the council proceedings. (2) a dues assessment of $7.50 per member for North American members for the 1952 calendar year, of $1.00 per member for members living outside of North America, and an assessment of $6.00 for the balance of the 1951 year (April-December) for the new members elected to membership at this meeting, this to include a 1951 subscription to the *Journal of Dental Research*. (3) Disbursements: For the 1951-1952 period up to December 31, 1952, disbursements were authorized as follows: (a) Secretary-treasurer's office, $675.00 as itemized in the council minutes. (b) Publication Committee, in the amount of $1,800.00 per year for the calendar years of 1951 and 1952 for expenses and honoraria as determined by the council. (c) Block subscriptions to the *Journal of Dental Research* for members at the rate of $6.00 per dues paying member ($0.50 additional for foreign subscriptions). The above actions were confirmed by unanimous vote.

A list of persons nominated to membership and certified by the council as having met all qualifications was read as printed elsewhere in these proceedings (page 530). On motion and by unanimous consent the constitutional provision for a formal written ballot was suspended and the secretary-treasurer instructed to cast a written ballot for the persons carried on that list to be elected to mem-

bership. On completion of this formality these persons were declared elected and entitled to all privileges of membership on payment of the first dues installment.

A proposed amendment to the by-laws was submitted by M. K. Hine, J. L. T. Appleton, H. J. Noyes, J. R. Blayney, and William Souder after approval by the council. It involves a change in item (C) of part (3) Exemptions, of Section B of the By-laws. The proposed revision reads as follows:

(C) The council is authorized to remit the dues of members of 25 or more years of membership in good standing, who have been retired from teaching or research positions or active practice and at their request in writing.

It was moved that the above named item (C) of the By-laws be repealed and replaced by the substitute submitted above. This motion was seconded and carried without a dissenting vote.

It was formally voted that all checks for withdrawals from association funds in the State Bank and Trust Co. of Evanston, Ill. be signed by any two of the following general officers: Edward H. Hatton, Secretary-treasurer; Leonard S. Fosdick, President; or Maynard K. Hine, President-elect.

All actions of the council as submitted in the form of the council minutes were approved by motion and vote, and the council authorized to act for the associations for the period between the 1951 and 1952 general meetings.

Reports were then received from the editor and business manager of the Publication Committee in the form made to the Board of Editors followed by a résumé of the proceedings of the meeting of the Board of Editors. On motion these reports were approved and ordered printed in the proceedings and the Publication Committee highly commended for the general excellence of the 1950 volume of the *Journal of Dental Research*.

The secretary-treasurer read a letter from Dr. Gies with reference to his recent illness and recovery and was instructed to convey to Dr. Gies the association's warm and sincere interest with congratulations for his return to good health and his usual activities. The loss of the following members by death since the last formal announcement was reported: Harry Bear, Thomas Cowling, Richard D. Dean, A. W. Ellis, Percy R. Howe, Leon R. Kramer, Warren C. Oxner. At President Bibby's request the audience stood briefly with bowed heads in respect and in grateful tribute to these former members.

A vote of appreciation followed for the members of the association who contributed to the success of this meeting, to the ladies who acted as registration clerks, to Dr. Hine and Richard Scott of the University of Indiana for the splendid projection service and to the members of the French Lick staff. President Bibby then addressed the association and at the close of his remarks he called the newly elected general officers to the space before the speaker's platform, formally installed them into their respective offices and then turned the gavel of authority over to the incoming president, L. S. Fosdick, who thereupon presented his inaugural remarks. This closed the evening session and the association adjourned its executive activities till the close of the Monday morning scientific session.

Third Session, Monday, 11:45 A.M., March 19, 1951

Meeting called to order by President Fosdick who, after calling for new business from the floor asked for a report of the Monday morning council session; this report was made and approved as shown in the council minutes. There being no other business the 1951 meeting of the association was declared adjourned, sine die.

D. OFFICERS AND MEMBERS ELECTED

ACTIVE OFFICERS: President—*Leonard S. Fosdick*, Northwestern University. President-elect—*Maynard K. Hine*, University of Indiana. Vice-president—*Francis A. Arnold, Jr.*, National Institute for Dental Research. Secretary-treasurer—*Edward H. Hatton*, Northwestern University. Trustee for a five-year term—*Leuman M. Waugh*, New York.

MEMBERS ELECTED: Alabama—*Heyl G. Tebo*, University of Alabama. California—*Louis J. Baume* and *H. Henry Weisengreen*, University of California. District of Columbia—*George W. Burnett*, Army Medical Service Graduate School; *Robert J. Nelsen*, National Bureau of Standards; *Richard W. Tiecke*, Armed Forces Institute of Pathology. Illinois—*Richard Q. Blackwell* and *Gordon H. Rovelstad*, Northwestern University and *John H. Manhold*, Great Lakes Naval Center. Indiana—*Ralph E. McDonald*, University of Indiana. Maryland—*W. V. Charter*, Bureau of Medicine, U. S. N.; *Robert B. Wolcott*, Naval Dental School; *Merrill G. Wheatcroft*, Naval Medical Research Institute. Massachusetts—*Jack Bloom*, Beth Israel Hospital; *Frederick W. Kraus, Vincent F. Lisanti, Frederic R. Shiere* and *Algirdas Yurkstas*, Tufts College. Michigan, *Harrison R. Hunt*, Michigan State College; *D. B. Mahler*, University of Michigan. Minnesota—*Dwight S. Chernausek*, University of Minnesota. Missouri—*Jerome Grosby*, St. Louis University and *John R. Ring*, Washington University. New York—*Melvin H. Amler*, New York University; *Alfred J. Asgis*, New York University; *Herbert J. Bartelstone*, National Institute for Dental Research; *Sidney Benzer*, New York University; *Sibyl L. Frank*, Cosmos Dental Products, Inc.; *Marvin Jacobson; H. H. Neumann*, Columbia University; *Robin M. Rankow*, Presbyterian Hospital; *Harold Sherman*, Columbia University; *William B. Savchuck*, National Institute for Dental Research; *Leo Zach*, New York University. Texas—*Harry M. Crawford*, Baylor U. Wisconsin—*Obed H. Moen*, Trustee A. D. A.; *Gunnar Ryge*, Marquette U. South Africa—*Bertie Cohen*. South America—*Julio C. Turell*. Total, 39.

E. MEMBERS VOTING AT THE ANNUAL ELECTION*

Agnew, Aisenberg, Allen, G. M. Anderson, Armstrong, Arnim, Arnold, Atkinson, Bagnall, Barr, Bauer, Bavetta, Bender, Bhaskar, Bibby, Bier, Blayney, Blumenthal, Boucher, Boulger, Boyle, Bradel, Brand, Brandhorst, Brashear, Brauer, Broadbent, Brodie, Brodsky, Brucker, Bunting, Burrill, Canby, Carr, Caul, Chase, Cheyne, Chilton, Clark, Clawson, Clough, M. M. Cohen, Costich, Cox, Coy, Cross, Crowley, Dahlberg, Dale, Darlington, H. T. Dean, Deatherage, Dickson, Dietz, Dobbs, Dragiff, Dressell, Dummett, Easlik, L. F. Edwards, F. C. Elliott, Ellis, Elvove, I. A. Epstein, Erikson, Everett, Fairbank, Fisher, Fleming, Fosdick, Fox, Freeman, Frey, Gafafer, Gamboa, Gilda, Ginn, Glass, H. J. V. Goldberg, M. A. Goldberg, Goldman, Goodsell, Grossman, Gurley, Hadjimarkos, Hadley, Hahn, Harrison, Hatch, Hensel, Higley, T. J. Hill, Hodge, Hooper, Hubbell, Hughes, Ireland, James, Jarabak, Jasper, Jay, W. A. Jordan, Kesel, Keyes, D. A. Keys, Khalifah, Kimball, King, Kitchin, Klaffenbach, Klatsky, Kniesner, Knighton, Koser, Kurth, Lalonde, Lasater, Law, Lefkowitz, Lewis, Lieban, Lieber, Linghorne, Losch, Lovestedt, Lowery, Lowry, Lund, Lynch, Macdonald, Main, Manly, Mann, Margolis, Marshall-Day, Martin, Mason, Massler, McCrea, Merkeley, Mercado, Merritt, Mershon, Midgley, Milhon, Millberry, S. C. Miller, Mitchell, A. W. Moore, Mary Moore, Norman Moore, T. E. Moore, Anna Morse, Fred W. Morse, A. H. Mueller, Emil Mueller, H. I. Myers, Nagle, Nelson, Neuwirth, H. J. Noyes, Nuckolls, Nylander, Oblatt, O. A. Oliver, Orban, Orland, Paffenbarger, Permarr, Peyton, Pfeiffer, Phatak, Phillips, Podolin, Prinz, Randall, Rapp, Rault, Revesez, H. B. G. Robinson, Rittershofer, Romnes, Rosenstein, Ruzicka, Ryder, Sarnat, Schantz, Scherer, Schoonover, Schour, Schroff, Seltzer, Shaner, Shaw, Shell, Sicher, Simonds, Skinner, Sloman, Smyd, Snyder, Sobel, Sognnaes, Sorrin, Stafne, Steadman, Steinmeyer, Stephan, T. D. Stewart, H. A. Swanson, W. F. Swanson, Swartz, J. T. Sweeney, Swenson, Taber, Tainter, N. O. Taylor, Thoma, B. O. A. Thomas, H. S. Thompson J. R. Thompson, Tiblier, Tilden, Van Huysen,

*By accident identity of some ballots was lost.

Vedder, Volker, Volland, Wach, Waggener, Wainwright, Wallace, Walter, Waldron, Ward, Wassermann, Waugh, Wessels, Wessinger, Wheeler, Wilbur, Willett, Williams, Wise, F. J. Wolfe, Wright, Zipkin. Total, 246.

F. MEMBERS IN ATTENDANCE AT ONE OR MORE SESSIONS, LISTED BY SECTIONS

Ann Arbor—Bunting, Kerr, Peyton. *Baltimore*—Dobbs, Hahn, McCrea, Robinson. *Boston*—Cohen, Dunning, Glass, Glickman, Greep, Manly, Marjerison, Marshall-Day, Nizel, Shaw, Sognnaes, Waldo, Zander. *Chicago*—Bhaskar, Blackwell, Blayney, Brodie, Burstone, Engel, Fosdick, Freeman, Hatton, Hill, Jarabak, Kesel, Koser, Krashner, Lundquist, Martin, Oriand, Rapp, Romnes, Schram, Skinner, Teuscher, Thompson, Tilden, Tylman, Wach, Wainwright, Wallace. *Cleveland*—Bruckner, Burwasser, Chase, Hill, Pearlman, Wylie. *Columbus*—Boucher, Kitchin, Robinson, Spangenberg. *Detroit*—Patterson. *Indianapolis*—Drexel Boyd, T. M. Boyd, Hall, Hine, Muhler Phillips, Shafer, Van Huysen. *Iowa City*—Boyd, Bryan, Smith. *Kansas City*—Ennever, Moore, Myers, Warner. *Louisville*—Burrill, Wilbur. *Minnesota*—Armstrong, Clark, Crawford, Lovestedt, McBride, Mitchell. *Nebraska*—Keys, Hooper. *New Orleans*—Peterson. *New York*—Bevelander, Butcher, Goldsmith, Hemley, Karshan, Levy, Shapiro, Taylor. *Oregon-Washington*—Law, Noyes, Thomas. *Philadelphia*—Appleton, Boyle, Burkett, Cobe, Crowell, Gable, Taylor, Williams. *Pittsburgh*—Cox, Harman, Strader, Swanson, Van Kirk. *Richmond*—Clough, Forbes, Kreshover, Lyons. *Rochester*—Brudevold, Bibby, Costich, Dale, Goldberg, Hein, Hodge. *San Francisco*—Agnew, Becks, Epstein, Fleming, Hughes, Scrivener, Sloman, Wylie. *St. Louis*—Allen, Boling, Brady, Brandhorst, Main, Rosebury. *Southern California*—Bavetta, Simmons. *Tennessee*—Ginn. *Toronto*—Ellis. *Washington*—Arnold, Bernier, Caul, Dean, Dickson, East, Hampp, Knutson, Likens, McClure, Oartel, Paffenberger, Parke, Pfeiffer, Rault, Rogosa, Schoonover, Scott, Souder, Stephan, Swaney, Sweeney, Zipkin.

Not members of a section—Brauer, Coleman, Finn, Hayes, Knighton, Lazansky, Ludwick, Molnar, Moore, Nelson, Reichborn-Kjennerud, Robinson, Schantz, Smyd, Volker, Wessinger. Total 179.

Attendance of New Members, Elected March 18, 1951. Bartelstone, Burnett, Hunt, Kraus, Lisanti, Nelsen, Ring, Rovelstad, Ryge, Savchuck, Tebo, Tiecke, Wheatcroft, Yurkstas. Total, 14.

Total registered attendance, including guests, 389.

GROWTH AND ACID PRODUCTION BY ORAL LACTOBACILLI IN THE PRESENCE OF VARYING AMOUNTS OF REQUIRED VITAMINS

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RECENT studies[1, 2] of vitamin requirements of oral lactobacilli have shown that the needs of many cultures can be met by supplying from 4 to 6 of the B vitamins. In a series of 26 such cultures studied in this laboratory it was found[1] all required biotin, nicotinic acid, pantothenic acid, and either riboflavin or thiamine; some also required pteroylglutamic acid (folic acid), vitamin B_6, or both vitamin B_6 and pteroylglutamic acid. These last 2 compounds at times accelerated growth when not an absolute requirement. In the series of 26 cultures examined, an absolute need for vitamin B_{12}, thymidine, phosphorylated vitamin B_6, or unidentified factors was not encountered. However, it is known that some oral lactobacilli need one or more of these substances,[2] as do occasional lactobacilli obtained from other sources.[3]

Since a suboptimum amount of one required vitamin limits both growth of the microorganism and the products of cell metabolism, even when all other vitamin and nutritive needs are fully met, the question arises as to how much acid would be produced by oral lactobacilli when any one of the required vitamins happens to be present in only small quantities. The usual studies of vitamin requirements do not supply much information on this point since often the vitamin is added somewhat in excess in the endeavor to secure optimum nutritive conditions for abundant growth. However, the use of microorganisms for vitamin assay procedures and especially the use of certain strains of lactobacilli of the *L. casei* and *L. arabinosus* types has supplied some information of the metabolic response in relation to the amount of needed vitamin in the environment, at least for the cultures which have now become more or less standard for use in microbiological assay procedures.

In the present work particular attention was devoted to the amounts of each vitamin capable of supporting acid production at a level which might be significant with respect to dental caries. The lactobacilli employed were from the collection of cultures previously used in the study of vitamin requirements and had been isolated originally either from saliva of persons with active caries, from plaques, or from cavities.

METHODS

Basal Medium.—The basal medium used for most of the work was the same as that previously employed[1] and consisted of 0.5 per cent acid-hydrolyzed purified casein fortified with several amino acids, 1.0 per cent glucose, 0.6 per cent

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sodium acetate, and inorganic salts including 0.2 per cent phosphate. It was prepared and tubed in such amounts that when diluted by the addition of sterile vitamin solutions the total volume was 10 ml. per tube, and this volume contained the specified amounts of ingredients. The pH was adjusted to 6.8 before autoclaving and was found to be 6.6 to 6.7 after autoclaving. Any departures from the usual composition of the medium or procedure are noted at appropriate places in connection with the results presented later.

Vitamins.—Solutions of each vitamin were prepared separately, sterilized by autoclaving or by filtration where necessary, and added aseptically to the basal medium. To test the limitations upon growth and acid production caused by suboptimum amounts of one vitamin, it is necessary to have all other needed vitamins present in ample amounts, otherwise the several resultant deficiences would not permit a clear interpretation of results. Accordingly, when graded quantities of one vitamin were added to a series of tubes of the basal medium, all other needed vitamins were supplied in an amount sufficient for fullest growth.

Cultures Used.—Several strains from the laboratory collection of oral lactobacilli were employed. These cultures were kept in stock in the laboratory in tomato juice-yeast extract medium, transferring approximately once a month with interim storage in the ice box. A few cultures with the heterofermentative type of sugar metabolism were included in the study, though most of them were of the homofermentative type. The latter produce lactic acid almost quantitatively from glucose while the former produce only about half as much lactic acid but greater amounts of other products, including carbon dioxide.

Inoculation of Media.—As a rule 24-hour cultures were used. These were made from the stock cultures and usually 0.001 ml. of culture, secured by dilution in buffered saline, was used to inoculate each tube of a series.

Growth and Acid Production.—Incubation was at 37° C. The response of the lactobacilli to different vitamin levels was followed by (1) measuring turbidity of the cultures in a Lumetron colorimeter with a 650 mμ filter, (2) determinations of pH made with a Leeds and Northrup Universal pH Indicator (model 7661) and (3) by titration of 10 ml. of culture with N/10 sodium hydroxide. Duplicate tubes were used and a test was repeated at least once and frequently two or more times.

PRELIMINARY EXPERIMENTS ON THE INCUBATION PERIOD AND MODIFICATION OF THE BASAL MEDIUM

Some early experiments were designed to throw light on certain questions concerning (1) length of the incubation period and (2) the effect of different amounts of nitrogenous and energy-yielding foodstuffs in the basal medium. The results of these tests are presented first. Concerning length of the incubation period, our earlier experience[1] with these cultures showed that most of them attained maximum growth, as shown by turbidity, in 3 or perhaps 4 days at 37° C. A few grew more slowly unless purines and pyrimidines were used as a supplement to the vitamins in the basal medium.

Fig. 1 shows the response in terms of pH levels of a typical culture to several amounts of nicotinic acid at different periods of incubation. This culture responds to the vitamin supplement rather promptly and is not one of those needing purines, pyrimidines, or other substances for reasonably prompt development. The amounts of 0.1 and 1.0 micrograms of nicotinic acid per ml. are sufficient for maximal change in pH values under the conditions of

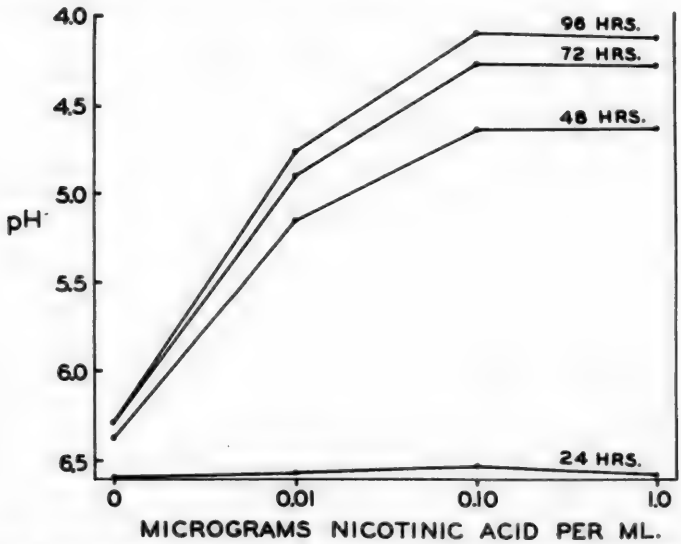

Fig. 1.—pH in relation to time of incubation at 37°C. and concentration of nicotinic acid. The casein digest basal medium is supplemented with all other needed vitamins in ample amounts. Culture 1009.

these tests, while 0.01 microgram is not sufficient for maximal response. The highest acidity (lowest pH value) was attained in either case after 4 days, although the 3-day value was almost as great. After 24 hours, acid production was barely detectable. Growth of the same culture as expressed by turbidity or titratable acid is not shown in the figure, but followed much the same pattern with the exception that maximal turbidity was attained at 3 days while titratable acid showed some increase between the third and fourth day.

Additional tests of the same sort were made with several other cultures and also with biotin as the limiting vitamin. In some cases a 72-hour incubation period resulted in about the same values as at 96 hours. Prolongation of the incubation period to 7 days was of no advantage except in the case of the few slower growing cultures. For the current investigation an incubation period of 3 or 4 days, usually 4, was chosen since the majority of our cultures attained maximal or practically maximal growth and acidity within this time.

In another comparative experiment the amount of casein hydrolyzate in the basal medium was increased from 0.5 to 1.0 per cent and glucose from 1.0 to 2.0 per cent. The amounts of all other ingredients of the medium remained

the same, including the phosphate and sodium acetate. Acid production in the two media with graded amounts of riboflavin, which was required by this culture, is shown in Fig. 2. With definitely suboptimum levels of the vitamin there was not much difference in acid production between the two media, whether expressed in terms of pH or as titratable acid. However, in the pres-

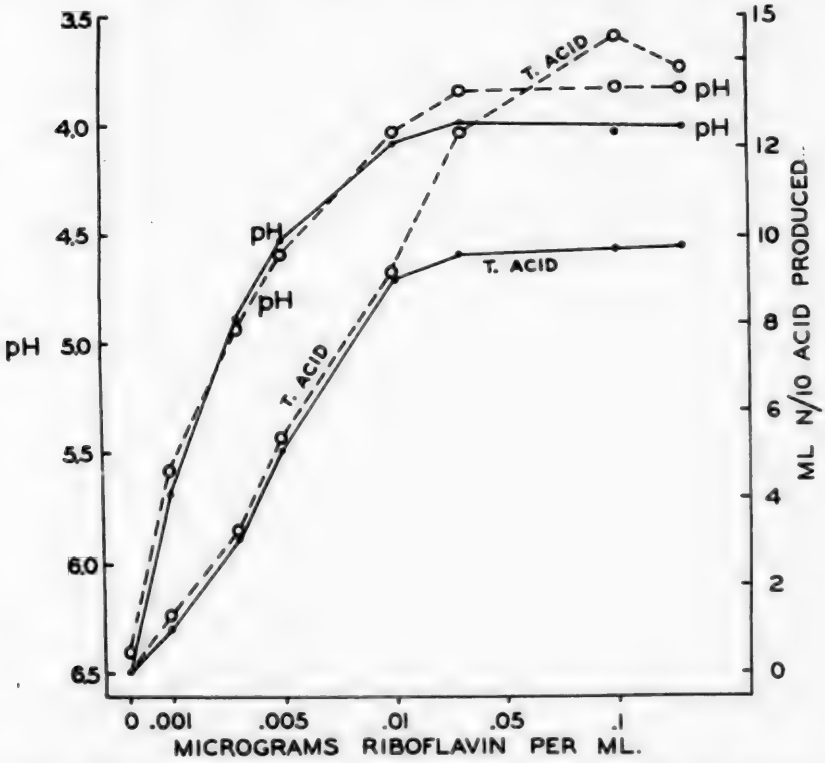

Fig. 2.—Effect of casein digest and glucose concentration upon pH and titratable acid in the presence of varying amounts of riboflavin. Other vitamins are present in amounts sufficient for maximal growth. Lactobacillus 1012, 4 days at 37°C.
———— indicates 0.5 per cent casein digest and 1.0 per cent glucose.
---------- indicates 1.0 per cent casein digest and 2.0 per cent glucose.

ence of sufficient riboflavin to support optimal growth, somewhat higher acidities were reached in the richer medium. Here the extra supply of food materials in the form of amino acids and sugar evidently was sufficient to support a somewhat greater cell population, with consequent increased acid production. Maximum turbidity figures for culture 1012 at the peak acidities shown in Fig. 2 were 77 to 78 in the 0.5 per cent casein hydrolyzate—1.0 per glucose medium, and 84 to 85 in the medium with these concentrations of foodstuffs doubled.

Similar results in comparison of the two basal media were secured with another culture with graded amounts of riboflavin, and also with two cultures

in the presence of graded amounts of biotin. These results are not shown in detail but in each instance the peak acidity was higher in the presence of the greater quantity of foodstuff.

In the following experiments with graded amounts of vitamins it was decided, in spite of the somewhat higher values with the richer medium, to maintain the level of casein digest and sugar at 0.5 and 1.0 per cent, respectively. Sufficient food materials to support the highest possible level of cell population might be present only rarely or intermittently under natural conditions. It is recognized, of course, that the same may also be true of the amounts selected for use. Nevertheless, it seemed reasonable to avoid the maximum of amino acids and sugar which yielded the highest possible values, while at the same time it was important to have available sufficient amounts of such food substances to support quite extensive cell metabolism and growth. This is necessary in order to measure adequately the effect of graded amounts of vitamins.

RESULTS OF TESTS WITH SUBOPTIMUM LEVELS OF VITAMIN

The limitations imposed upon growth and acid production by suboptimum levels of one required vitamin in the presence of ample amounts of other needed vitamins are shown in the accompanying tables. Table I summarizes the

TABLE I

EFFECT OF GRADED AMOUNTS OF RIBOFLAVIN ON ACID PRODUCTION AND GROWTH

AMOUNT OF VITAMIN, MICROGRAMS PER MILLILITER	pH			TITRATABLE ACID*			TURBIDITY†		
	LACTOBACILLUS			LACTOBACILLUS			LACTOBACILLUS		
	46	1009	1012	46	1009	1012	46	1009	1012
0	6.45	6.47	6.50	0.2	0.1	0.1	1	0	0
.001	5.74	5.72	5.67	1.0	1.0	1.0	7	3	3
.003	5.03	4.92	4.89	2.9	2.7	3.1	24	16	16
.005	4.58	4.61	4.52	5.2	4.5	5.1	35	28	27
.01	4.16	4.04	4.08	9.1	9.3	9.0	64	60	56
.03		4.01	3.98		9.7	9.6	75	74	77
.1	4.15	4.03	4.01	9.3	9.8	9.7	82	74	78
.3	4.12	4.01	4.00	9.6	9.8	9.8	82	74	77
1.0	4.29	4.01	4.00		9.8	9.7	80	73	78

The amounts of riboflavin shown in the left-hand column were added to a casein digest-glucose-salts basal medium which contained all other needed vitamins in amounts sufficient for maximal growth. The amount of riboflavin is therefore the limiting factor here.

All cultures were incubated 4 days at 37°C.

*In terms of N/10 NaOH per 10 ml. culture.

†0 = clear tube, higher figures indicate increasing turbidities; 60 to 80 represents a very turbid culture or "heavy growth."

response of several typical homofermentative oral lactobacilli, which require riboflavin, to varying amounts of this vitamin. Very small quantities of the vitamin serve to support light growth as shown by turbidity and the production of acid. In the presence of 0.003 microgram per milliliter a pH of approximately 5.0 is attained, with 0.01 to 0.03 microgram per milliliter a pH of about 4.0 to 4.16 is reached and titratable acid and growth are maximum or close to it.

Results with graded amounts of nicotinic acid are shown in Table II. In the presence of amounts as small as 0.0005 microgram per milliliter slight acid

TABLE II

EFFECT OF GRADED AMOUNTS OF NICOTINIC ACID ON ACID PRODUCTION AND GROWTH

AMOUNT OF VITAMIN, MICROGRAMS PER MILLILITER	pH			TITRATABLE ACID			TURBIDITY		
	LACTOBACILLUS			LACTOBACILLUS			LACTOBACILLUS		
	46	1009	1012	46	1009	1012	46	1009	1012
0	6.05	6.10	6.07	0.55	0.6	0.55	7	7	6
.0005	5.92	5.98	5.92	0.7	0.7	0.7	11	10	8
.001	5.78	5.91	5.81	1.0	0.9	0.9	15	12	12
.003	5.50	5.55	5.39	1.5	1.6	1.6	23	23	27
.005	5.28	5.26	5.09	2.5	2.2	2.5	35	32	37
.01	4.83	4.87	4.70	3.9	4.0	4.8	55	50	55
.03	4.37	4.44	4.10	7.4	6.5	9.2	69	66	73
.05	4.22	4.32	4.06	8.6	8.5	10.0	73	69	75
.1	4.20	4.33	4.06	9.2	8.2	9.8	75	71	76

Cultures 46 and 1009 were incubated 3 days, culture 1012 4 days at 37°C.
Other conditions and determinations similar to Table I.

production and growth over that shown in the control tubes could be detected. Levels from 0.005 to 0.01 microgram per milliliter supported sufficient cell metabolism and sugar breakdown to attain pH 5.0. With 0.03 to 0.1 microgram per milliliter pH values of 4.4 to about 4.0 were reached. In the case of pantothenate (Table III) only 0.001 to 0.003 microgram per milliliter was needed for culture 1009 to reach pH 5.0. Heterofermentative cultures such as 1013 produce less acid. Amounts of 0.03 to 0.1 microgram of pantothenate per milliliter supported abundant growth and an acidity of pH 4.0 to 4.1 in the case of the homofermentative culture 1009. The figures for pantothenate are in line with those reported by Hill and Kniesner[4] who found that from 0.04 to 0.06 microgram per milliliter of medium supported maximal acid production of the majority of their 9 strains of oral lactobacilli. In the presence of limiting amounts of biotin (Table IV) pH values of nearly 5.0 were reached with a concentration of 0.00001 microgram per milliliter, while 0.0001 to 0.0003 microgram supported full growth and acid production for the two types of lactobacilli under the conditions of these tests. Biotin is very potent physiologically and only minute quantities are needed to support maximal growth.

TABLE III

EFFECT OF GRADED AMOUNTS OF CALCIUM PANTOTHENATE ON ACID PRODUCTION AND GROWTH

AMOUNT OF VITAMIN, MICROGRAMS PER MILLILITER	pH		TITRATABLE ACID		TURBIDITY	
	LACTOBACILLUS		LACTOBACILLUS		LACTOBACILLUS	
	1009	1013*	1009	1013	1009	1013
0	5.28	6.48	1.9	0.2	8	7
.0001	5.28	6.43	1.9	0.2	10	10
.0003	5.12	6.48	2.4	0.2	10	9
.001	5.07	6.47	2.7	0.2	12	7
.003	4.82	6.31	3.9	0.3	22	12
.01	4.42	5.77	5.2	1.0	44	29
.03	4.08	4.65	9.5	5.0	66	61
.1	4.03	4.65	9.6	5.0	73	60

Both cultures were incubated 4 days at 37°C.
*Lactobacillus 1013 is representative of the heterofermentative type of metabolism and produces less lactic acid from glucose.

TABLE IV

EFFECT OF GRADED AMOUNTS OF BIOTIN ON ACID PRODUCTION AND GROWTH

AMOUNT OF VITAMIN, MICROGRAMS PER MILLILITER	pH		TITRATABLE ACID		TURBIDITY	
	LACTOBACILLUS		LACTOBACILLUS		LACTOBACILLUS	
	1009	1013*	1009	1013	1009	1013
0	6.28	6.60	0.4	0	1	0
.000003	5.58	6.08	1.1	0.6	7	7
.00001	5.02	5.14	3.0	2.3	13	35
.00003	4.40	4.61	6.5	4.9	41	65
.0001	4.10	4.61	9.7	4.9	68	64
.0003	4.05	4.61	9.9	4.9	78	74
.001	4.04	4.60	9.8	4.9	79	67

Both cultures were incubated 4 days at 37°C.
*Lactobacillus 1013 is representative of the heterofermentative type of glucose metabolism.

Results for pteroylglutamic acid (folic acid), pyridoxamine hydrochloride, and pyridoxal hydrochloride are shown in Table V. In these tests growth of the lactobacilli in the control tubes, without added vitamin, was somewhat heavier than in the controls of previous tests. Perhaps certain substances in the casein digest basal medium supported growth to a limited extent in lieu of the vitamin. However, it is evident the respective vitamins are definitely needed for good growth of the strains used in these tests. In the case of pteroylglutamic acid, an amount as small as 0.00003 microgram per milliliter supported detectable growth and acid production over that of the control tubes, 0.001 microgram per milliliter supported heavy growth and pronounced acid production, and 0.01 to 0.1 gave maximum values. Indeed, there was little difference in the degree of acidity and the amount of growth with quantities of pteroylglutamic acid ranging from 0.003 to 0.1 microgram per milliliter; all were close to the maximum. Similar results were secured with two other strains of oral lactobacilli which are not shown in the table.

With pyridoxamine somewhat larger amounts were needed. Maximum acid production and growth were reached only in the presence of 0.3 microgram per milliliter, while 0.01 microgram caused a barely detectable response. In tests with another oral lactobacillus (not given in Table V) 1.0 microgram of pyridoxamine hydrochloride was needed for maximum growth and acid production. When pyridoxal hydrochloride was supplied the response occurred at lower levels than for pyridoxamine. For the culture shown in Table V distinctly increased growth and acid production over the control were evident at 0.001 to 0.003 microgram per milliliter. These effects became more pronounced with 0.005 microgram, and reached maximum or near maximum values with 0.03 to 0.1 microgram per milliliter. Another culture gave quite similar results. The pyridoxamine and pyridoxal used in the tests were both sterilized by filtration and added aseptically to the medium.

The response of different lactobacilli and of other microorganisms to the several related compounds with vitamin B_6 activity varies greatly. Most lactobacilli either do not respond or respond poorly to pyridoxine, they respond more readily to pyridoxamine, and often quite readily to pyridoxal. Some, however,

TABLE V

EFFECT OF GRADED AMOUNTS OF PTEROYLGLUTAMIC ACID, PYRIDOXAMINE HYDROCHLORIDE, AND PYRIDOXAL HYDROCHLORIDE ON ACID PRODUCTION AND GROWTH

AMOUNT OF VITAMIN, MICROGRAMS PER MILLILITER	PTEROYLGLUTAMIC ACID — LACTOBACILLUS 1009			PYRIDOXAMINE HYDROCHLORIDE — LACTOBACILLUS 46			PYRIDOXAL HYDROCHLORIDE — LACTOBACILLUS 46		
	pH	TITRATABLE ACID	TURBIDITY	pH	TITRATABLE ACID	TURBIDITY	pH	TITRATABLE ACID	TURBIDITY
0	5.60	1.3	11	5.50	1.3	13	5.25	1.8	8
.00003	5.30	1.8	17						
.0001	5.05	2.8	27						
.0003	4.78	3.9	41						
.001	4.17	8.6	72	5.51	1.3	13	5.17	2.1	11
.003	4.10	9.3	78	5.33	1.6	14	4.80	3.4	37
.005	4.10	9.4	80				4.41	5.2	60
.01	4.05	9.6	81	5.33	1.7	21	4.11	8.6	77
.03	4.04	9.5	81	5.10	2.5	26	4.03	9.3	84
.1	4.02	9.8	83	4.76	3.8	51	4.00	9.2	83
.3				4.10	10.6	84	4.01	9.7	84
1.0				4.10	10.4	85	3.98	9.8	85

Cultures were incubated 4 days at 37°C.

respond poorly to all of these substances and need a phosphorylated form of the vitamin. None of the cultures used in the current investigation fell into this last category. The activity of different microorganisms, including several lactobacilli, toward vitamin B_6 compounds has been studied especially by Snell, Rannefeld, Rabinowitz, and McNutt[5] and by Hendlin, Caswell, Peters, and Wood.[6]

Thiamine is needed for growth only by heterofermentative lactobacilli. In our experiments 0.01 to 0.03 microgram of thiamine per milliliter was sufficient for approximately maximum growth and acid production (Table VI), while 0.001 to 0.003 microgram supported a detectable response.

TABLE VI

EFFECT OF GRADED AMOUNTS OF THIAMINE HYDROCHLORIDE ON ACID PRODUCTION AND GROWTH OF HETEROFERMENTATIVE ORAL LACTOBACILLI

AMOUNT OF VITAMIN, MICROGRAMS PER MILLILITER	pH		TITRATABLE ACID		TURBIDITY	
	LACTOBACILLUS		LACTOBACILLUS		LACTOBACILLUS	
	41	1013	41	1013	41	1013
0	6.54	6.59	0	0	0	0
.001	6.48	6.59	0.1	0	2	1
.003	6.26	6.55	0.3	0.1	7	2
.005	6.04	6.44	0.5	0.2	12	7
.01	4.64	4.79	4.6	1.9	66	57
.03	4.62	4.69	5.0	4.9	66	70
.1	4.60	4.66	4.9	4.9	62	63
.3	4.60	4.66	4.8	4.9	63	70

Finally, it should be added that the use of combinations of the vitamins in the amounts in which they occur in saliva would be desirable and it is hoped that this work will be continued with such an objective in view. But such studies must await further information on the amounts of several of the vitamins in saliva.

SUMMARY

Vitamins needed for the growth of oral lactobacilli were each added in graded amounts to a basal medium of casein digest, glucose, and salts. When the amount of one needed vitamin was reduced, ample supplies of other needed vitamins and nutritive substances were present. The response of the lactobacilli was followed by measuring turbidity, pH, and titratable acid.

With allowance for some differences between individual strains, the following amounts of vitamins sufficed for maximal or practically maximal growth and acid production (about pH 4.0) under the conditions of these tests: in terms of micrograms per milliliter of medium, biotin 0.0003, nicotinic acid 0.05, calcium pantothenate 0.03-0.1, riboflavin 0.03, pteroylglutamic acid (folic acid) about 0.01, pyridoxamine hydrochloride 0.3 to 1.0, pyridoxal hydrochloride 0.03-0.3, and thiamine hydrochloride 0.01 to 0.03.

Smaller amounts of vitamins supported less growth and acid production, the response being usually in relation to the amount of vitamin supplied. In a general way, amounts of each vitamin approximately one-tenth of those speci-

fied above sufficed for attainment of about pH 5.0, except in the case of thiamine and the heterofermentative lactobacilli. Still smaller amounts supported detectable acid production.

REFERENCES

1. Koser, S. A., and Fisher, B. J.: Vitamin Requirements of Oral Lactobacilli, *J. D. Res.* **29**: 760, 1950.
2. Rogosa, M.: Personal Communication, 1950.
3. Kitay, E., and Snell, E. E.: Some Additional Nutritional Requirements of Certain Lactic Acid Bacteria, *J. Bact.* **60**: 49, 1950.
4. Hill, T. J., and Kniesner, A. H.: Nutritional Requirements of Oral Lactobacilli. I. Pantothenic Acid and Dextrose (d-Glucose), *J. D. Res.* **21**: 467, 1942.
5. Snell, E. E., and Rannefeld, A. N.: The Vitamin B_6 Group. III. The Vitamin Activity of Pyridoxal and Pyridoxamine for Various Organisms, *J. Biol. Chem.* **157**: 475, 1945.
   Rabinowitz, J. C., and Snell, E. E. : The Vitamin B_6 Group. XII. Microbiological Activity and Natural Occurrence of Pyridoxamine Phosphate, *J. Biol. Chem.* **169**: 643, 1947.
   McNutt, W. S., and Snell, E. E.: Pyridoxal Phosphate and Pyridoxamine Phosphate as Growth Factors for Lactic Acid Bacteria, *J. Biol. Chem.* **182**: 557, 1950.
6. Hendlin, D., Caswell, M. C., Peters, V. J., and Wood, T. R.: The Nature of the TJ Factor for Lactobacillus lactis Dorner, *J. Biol. Chem.* **186**: 647, 1950.

INFLUENCE OF AMMONIA AND OF UREA UPON L. ACIDOPHILUS 4646

I. Studies on Growth

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THE results of many studies have indicated that susceptibility to dental caries is associated with the appearance of relatively large numbers of lactobacilli in the saliva.[1-6] The nature of this association and its relation to the etiology of the disease is not clearly understood. There is, however, a popular belief that the elimination of lactobacilli from the oral cavity will result in cessation of caries activity and in the prevention of further attacks of the disease.

A review of the literature concerning the occurrence and possible significance of ammonia in saliva has been compiled by Kesel, O'Donnell, Kirch, and Wach.[8] The same authors have presented evidence[7, 9] which suggested that the growth of *L. acidophilus* 4646 was inhibited in vitro by small amounts of ammonia, under alkaline conditions. Because inhibition was not observed if the pH of the culture medium was below 7, they attempted to show that neither alkalinity nor the ammonium ions (NH_4^+) were responsible for the effects, but rather that free ammonia (NH_3) was the active factor. They showed further that ammonia inhibited the formation of acids in incubating mixtures of saliva and glucose and that urea in combination with the phosphate produced even more striking results. They have reported periodically upon the clinical aspects of their investigations.[7-11]

The possible clinical importance of urea in relation to dental caries was discussed by Stephan[12], who suggested that its hydrolysis by urease-containing oral microorganisms would result in the production of ammonia which could then neutralize acids formed on the tooth surface, and might prevent the pH from falling to the level below which dental enamel is rapidly dissolved.

Stephan demonstrated that strong solutions of urea (40 per cent or more) inhibited the drop in pH which normally occurs in the dental plaque after exposure of the plaque to glucose solution.[13] The inhibitory effect was demonstrated in vitro with plaque material scraped from the teeth, and also in situ by measuring the pH of plaques in the human mouth by means of a specially constructed antimony electrode. Lesser concentrations of urea produced only partial inhibition.

Clinical studies of the retardation of dental caries through the use of a dentifrice containing 45 per cent urea were reported by Stephan and Miller.[14] While the number of subjects was undesirably small, the effects of 16 months' usage of the dentifrice were remarkable in that, in several cases, there were no new lesions during this period, despite the fact that the caries process had been highly active during the preceding 18 months.

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Muntz and Miller[15], working with homogenates of plaque material in vitro, demonstrated that urea rapidly penetrated into their artificial plaques, apparently by simple diffusion. They found that exposure of the layered plaque to 50 per cent urea for 10 minutes inhibited nearly 90 per cent of the glycolytic activity observed upon subsequent exposure to 1 per cent glucose. They stated that the inhibition was apparently due to the protein-denaturing property of the urea.

Kesel, O'Donnell, Kirch and Wach[9] were interested in incorporating urea into a dentifrice in concentrations that would provide a substrate from which ureolytic oral bacteria might liberate ammonia. They did not use highly concentrated solutions because of the possibility of denaturing the enzymes responsible for ureolysis in saliva. Experiments in vitro with mixtures of saliva, glucose 2.5 per cent, ammonium phosphate 0.625 per cent and urea 0.375 per cent (final concentrations) demonstrated that lactic acid formation was not significantly retarded by either ammonium phosphate or urea alone, but that in the presence of both together in the stated concentrations no lactic acid was formed from the glucose during an incubation period of 48 hours. Furthermore, they showed that whereas large numbers of lactobacilli could be demonstrated in the mixtures at the beginning of the experiment, none could be grown out at the end of 24 hours. This phenomenon was attributed to the extremely large quantity of ammonia that appeared during the incubation period. They did not report any studies of the effect of this combined solution upon pure lactobacillus cultures.

In consideration of the small quantities of ammonia that were used, and of the common laboratory practice of employing ammonium salts to stimulate bacterial growth, the findings of Kesel, O'Donnell, Kirch, and Wach aroused interest apart from their possible significance in the control of dental caries.

According to Kesel, O'Donnell, Kirch and Wach,[7, 9] growth of the organism was inhibited and viability was negative after 72-hour incubation in a medium composed of "ordinary beef infusion broth," adjusted with disodium phosphate to pH 7.2, with the addition of 1 ml. of 5 per cent dibasic ammonium phosphate. The availability of free ammonia in this solution at pH 7.2 may be calculated from the Henderson-Hasselbalch equation.[16] The concentration of NH_3 after dilution of the 5 per cent ammonium phosphate with the broth is approximately 1 part in 200,000. It is unlikely that ammonia at this low concentration would be capable of exerting its inhibitory effect by inducing gross physico-chemical changes in the bacteria. Usually, substances which influence an organism in minute concentrations do so by affecting one or more enzyme systems.

The experimental studies to be described were designed to determine the effects of ammonia and of urea upon *L. acidophilus* 4646 with a view toward clarifying the manner in which these substances may affect its growth.

EXPERIMENTAL

Effect of Dibasic Ammonium Phosphate on Growth Rate.—The procedures of Kesel, O'Donnell, Kirch and Wach[17] were followed as closely as possible. "Ordinary beef infusion broth" was interpreted to correspond with nutrient

broth (Difco), and the medium was prepared as follows: nutrient broth powder (Difco) 8 Gm.; dextrose anhydrous (Merck) 20 Gm.; distilled water (approximately) 600 ml. One-tenth molar disodium phosphate was added to adjust the pH from 6.8 to 7.6. Approximately 150 ml. of phosphate solution per liter were usually required for this operation. The adjusted medium was diluted to 1000 ml., dispensed in suitable tubes, and autoclaved for 15 minutes at 15 pounds pressure.

L. acidophilus 4646 was subcultured daily in this medium by repeated 10 per cent inoculations from 24-hour cultures. Growth was not as rapid nor as luxuriant as in media with pH below 7.

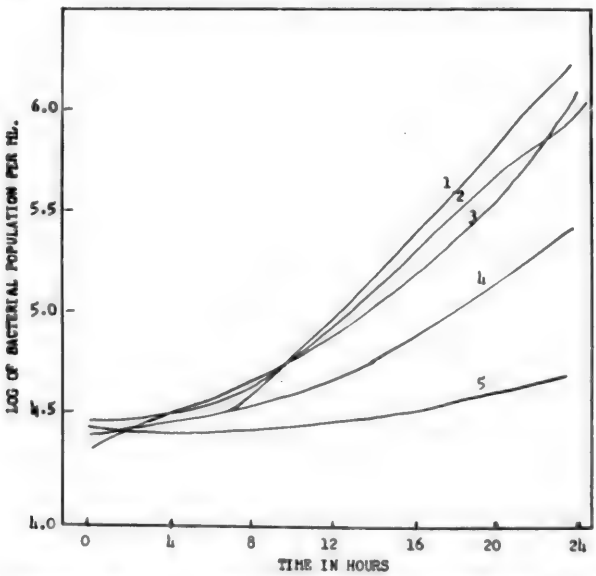

Fig. 1.—Growth curves of *L. acidophilus* 4646 in glucose-nutrient broth at alkaline pH with different concentrations of dibasic ammonium phosphate. (First 24 hours.) Series 1.- 0 mg.-N per ml.; 2.- 0.22 mg.-N per ml.; 3.- 0.57 mg.-N per ml.; 4.- 1.11 mg.-N per ml.; 5.- 1.98 mg.-N per ml.

Growth was estimated by the dilution plate technique. Samples were diluted serially with sterile saline solution, and 0.1 ml. of the proper dilution was spread over the surface of tomato juice agar plates. The colonies were counted after 4-day incubation at 37° C., and the number of bacteria per milliliter of the original culture was calculated.

Considerable experimentation demonstrated that the dilution plate method was subject to large errors, and that the estimation of the bacterial numbers by this technique would be enhanced by the use of several replicate counts of each specimen. Different counts of any one sample usually fell within the same logarithmic unit, and a more lucid conception of the results was obtained by treating the data logarithmically. All data represent the average of 3 replicates on each sample. In most cases the dilutions were such that 30 to 300 colonies appeared on each plate.

In the experiment represented in Figs. 1 and 2, 1 ml. of dibasic ammonium phosphate in serially increasing concentrations was added to 4 ml. quantities of glucose-nutrient broth. The tubes were inoculated with 0.1 ml. of a 1:100 dilution of a 24-hour culture of *L. acidophilus*. The cultures were incubated at 37° C., and samples were removed for counting, at the intervals shown. These samples were diluted as required and suitable dilutions were plated in triplicate. The pH of identical cultures was measured at intervals during the experiment. The ammonia concentration in the cultures at the beginning and at the end of the experiment was determined by steam distillation and titration, no significant changes were observed.

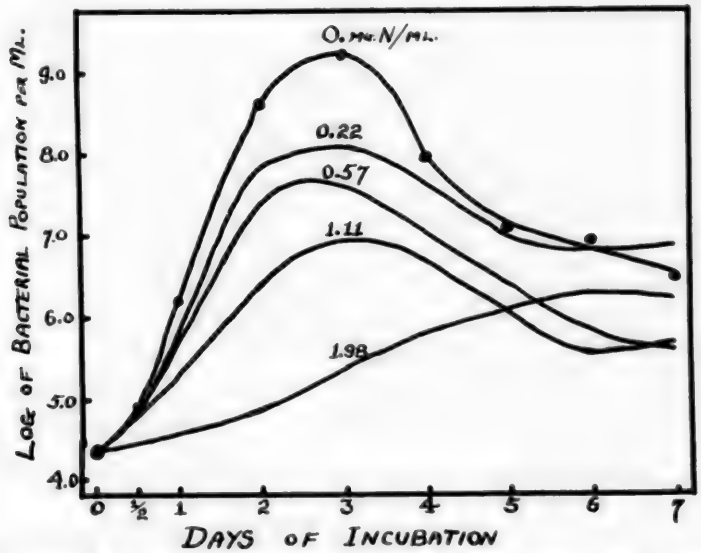

Fig. 2.—Growth curves of *L. acidophilus* 4646 in glucose-nutrient broth at alkaline pH with different concentrations of dibasic ammonium phosphate.

The depression of the rate and extent of growth was not due to initiation of a "lag-phase" (Fig. 1). While the peaks of the growth curves (Fig. 2) are successively lower with increasing salt concentration, it is noteworthy that even at a salt concentration equivalent to 2 mg. N per ml., the organisms continued to multiply for 7 days. This is in direct contrast with the reports of Kesel, O'Donnell, Kirch, and Wach,[7] who found no viable organisms after a 72-hour exposure to that quantity of ammonium phosphate, initially at pH 7.2. The initial and final pH values of the cultures are shown in Table I. It may be seen that the final pH was low in those cultures where the greatest growth occurred.

The inclusion of larger quantities of ammonium phosphate was accompanied by a corresponding elevation of the initial pH level, from pH 7.4 in the control to pH 7.7 in tube 4. The original ammonium phosphate solution was at pH 7.7, and it is evident that the differences noted are related to the amount of phos-

TABLE I

TYPICAL pH VALUES BEFORE AND AFTER INCUBATION OF L. ACIDOPHILUS IN GLUCOSE-NUTRIENT BROTH WITH ADDED DIBASIC AMMONIUM PHOSPHATE

TIME (DAYS)	CONTROL	0.22 MG.-N PER ML.	0.57 MG.-N PER ML.	1.11 MG.-N PER ML.	1.98 MG.-N PER ML.
0	7.4	7.5	7.6	7.7	7.7
8	4.3	4.4	4.7	5.7	7.1

phate added. While these differences are relatively small, their importance may be considerable since the variations occur close to the limit of alkalinity beyond which the organism does not readily multiply.[18]

To avoid this variation in pH, buffered solutions were employed in subsequent experiments, and were prepared by adjusting the dibasic salt solution with small quantities of an equimolar solution of the monobasic salt. The buffers were autoclaved before use. It was found that this procedure resulted in the loss of 3.6 per cent of the ammonia. In subsequent preparations of ammonium phosphate solutions to be autoclaved, a calculated excess of reagent was added to compensate for this loss. It was recognized that the foregoing procedure resulted in slightly higher phosphate concentrations in these solutions. The actual quantity of ammonia-nitrogen present after autoclaving was verified by steam distillation and titration.

Variation of the Cation.—Having established that ammonium phosphate was inhibitory to the growth of *L. acidophilus 4646,* it was essential to learn whether phosphates of other monovalent ions also possessed this feature.

Broth was prepared and adjusted to pH 7.6 as in the previous experiment. Ammonium, sodium, and potassium phosphate buffers were prepared by acidifying 50 ml. portions of each dibasic salt to pH 7.6 with the corresponding monobasic salt. The cation concentrations were altered only slightly by this procedure. The solutions were autoclaved for 15 minutes at 15 pounds pressure. These buffers were 0.38 Molar, corresponding with 5 per cent dibasic ammonium phosphate (10.6 mg. N per milliliter). The ammonium phosphate buffer was prepared so that 10.6 mg. N per milliliter were present *after* autoclaving. One ml. of each buffer was added to 4 ml. portions of broth which were then inoculated in the manner described. In addition, 0.5 ml. of sodium and 0.5 ml. of potassium buffer were added to a fourth series of tubes, and a water control was included. Counts were made at once and after a 48-hour incubation. The pH readings were made on a Cambridge meter. The results are shown in Table II. Sodium, ammonium, and potassium phosphates were inhibitory to growth,

TABLE II

THE GROWTH OF L. ACIDOPHILUS 4646 IN 0.08 M NH_4^+, Na^+, AND K^+ PHOSPHATE BUFFERS

	CONTROL (UNBUFFERED)		1. NH_4^+		2. Na^+		3. K^+		4. $Na^+ - K^+$	
TIME HRS.	LOG OF COUNT	pH	LOG OF COUNT	pH	LOG OF COUNT	pH	LOG OF COUNT	pH	LOG OF COUNT	pH
0	4.3	7.5	4.3	7.7	4.3	7.8	4.3	7.8	4.2	7.7
48	9.9	6.9	5.0	7.4	<2*	7.6	7.1	7.5	6.4	7.4

*No colonies grew on plates inoculated with broth diluted to 10^{-6}, 10^{-4}, or 10^{-2}.

in that order of intensity. The degree of inhibition shown by the sodium-potassium mixture was between that of those cations taken alone. Because the broth was originally adjusted with sodium phosphate, the actual concentration of sodium phosphate was somewhat higher than the others.

Variation of the Anion.—To determine the effects of anions other than phosphate, buffer solutions were prepared having the same molar cationic concentration as 5 per cent dibasic ammonium phosphate. The chloride, citrate, sulfate, and acetate salts of ammonium were employed. Equimolar solutions of the corresponding potassium or sodium salt were used as controls. The pH was adjusted to 7.6 with NaOH or HCl as required. The solutions were added to broth in the manner described, and the cultures were incubated for 48 hours. The results appear in Table III, and show that all of the sodium salts were

TABLE III

EFFECT OF VARIOUS SALTS UPON THE GROWTH OF L. ACIDOPHILUS 4646

TIME	H_2O CONTROL	CHLORIDES		CITRATES		SULFATES		ACETATES	
		NH_4^+	Na^+	NH_4^+	Na^+	NH_4^+	Na^+	NH_4^+	Na^+
	LOG COUNT	LOG COUNT	LOG COUNT	LOG COUNT	LOG COUNT	LOG COUNT	LOG COUNT	LOG COUNT	LOG COUNT
0	4.3	4.3	4.3	4.2	4.3	4.3	4.2	4.2	4.3
48	9.6	8.0	3.1	3.2	1.0	6.9	8.3	9.6	2.7
pH at 48 hrs.	6.2	6.9	7.1	7.3	7.4	6.9	6.8	6.5	7.3

inhibitory. It should be recalled that the concentration of sodium in these tubes was higher than the cation concentration of the other salts, since approximately 0.016 M sodium phosphate was present in the broth itself and this difference might have contributed to the intensity of the effect. Ammonium citrate was the only other test compound that produced marked inhibition. The remaining salts had comparatively little activity.

Effect of pH.—From the preceding experiments, it appeared that the inhibitory salts were those which were capable of exerting some buffer action in the range pH 7.0, namely phosphates and citrates. The data indicated that, above pH 7.0, growth of the organisms was suppressed in the presence of a relatively high concentration of buffer, regardless of the constituent cations that were tested. The pK_a of each active compound was such that a considerable barrier was presented to acidification of the medium by the metabolic acids produced by the bacteria. When aliquots of the inhibitory broth-buffer mixtures were titrated with 0.10 N H_2SO_4, buffer curves were obtained which substantiated this suggestion.

Thus, phosphate ($pK_2 = 6.7$),[16] and citrate ($pK_3 = 6.4$)[16] inhibited growth, whereas acetate ($pK_a = 4.7$),[16] chloride, and sulfate did not. The buffer capacity of ammonia (pK_a ca. 9.3) is virtually exhausted, toward acid, at the pH level of these experiments.

Weiss and Rettger[18] found that the optimum growth range for their strains of *L. acidophilus* lay between pH 5.8 and 6.6, with limits at 4.6 and 6.8. While strain 4646 survives above this upper limit, it appeared probable that the in-

hibitory properties of the test compounds depended upon their ability to sustain the alkalinity of the medium, thereby maintaining an environment that is unfavorable for optimal multiplication of the cells.

To test this hypothesis, broth was prepared without Na_2HPO_4, and portions were adjusted carefully with NaOH and HCl to pH 7.5 and 6.5 respectively. Ammonium and potassium phosphate buffers, 0.38 M, were adjusted to those pH levels and autoclaved, and were added to the broth in routine fashion. Ammonia-nitrogen in the broths was estimated by Nesslerization. The tubes contained a final volume of 5 ml., and were inoculated with one drop of a 48-hour culture of *L. acidophilus* 4646. The entire experiment was performed in sextuplicate.

Counts were made at the beginning of the experiment and again after a 30-hour incubation. At this time the pH values of the buffered broths had not changed. The data which were obtained are shown in Fig. 3. At pH 6.5, neither potassium nor ammonium phosphate produced significant inhibition, although the ammoniated broths showed somewhat less growth than the other two. At pH 7.5, inhibition was produced both by K^+ and NH_4^+. However, the potassium salt did not depress growth as drastically as did the ammonium salt.

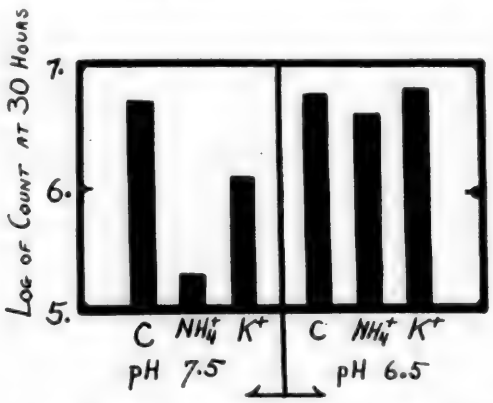

Fig. 3.—Effect of NH_4^+ and K^+ phosphates upon growth of *L. acidophilus* 4646 in glucose-nutrient broth above and below pH 7.0. Columns represent average count of 6 tubes. C.—unbuffered control. NH_4^+—0.08 M ammonium phosphate buffer present. K^+.—0.08 M potassium phosphate buffer present.

Since the pH of the buffered cultures did not drift appreciably during the experiment, it appeared that at pH 7.5, ammonium exerted a specific effect under the conditions employed, and that alkalinity alone did not adequately explain the phenomena.

Kesel, O'Donnell, Kirch and Wach[7] had stated that alkalinity did not explain the inhibition which they observed. The basis of the statement was an experiment in which a solution of ammonium acetate containing "5.6 mg. per ml." was added to one portion of their broth, and a solution of sodium acetate "having the same pH—7.2" was added to a similar portion. After inoculation and incubation, growth was evident in the tube containing sodium acetate, but not in the tube containing ammonium acetate.

However, we found that a solution of ammonium acetate (Baker) containing 5.6 mg. N per ml. (0.4 Molar) was at pH 6.9, and not 7.2 as reported. Furthermore, a solution of sodium acetate (Merck) at pH 7.2 was only 0.013 Molar with respect to the salt. The molar concentration of the ammonium acetate solution used by Kesel and his co-workers may possibly have been 30 times as high as that of their sodium acetate solution. Therefore, the effects observed in their experiment might have been due either to alkalinity or to unequal buffer activity at unfavorable pH levels.

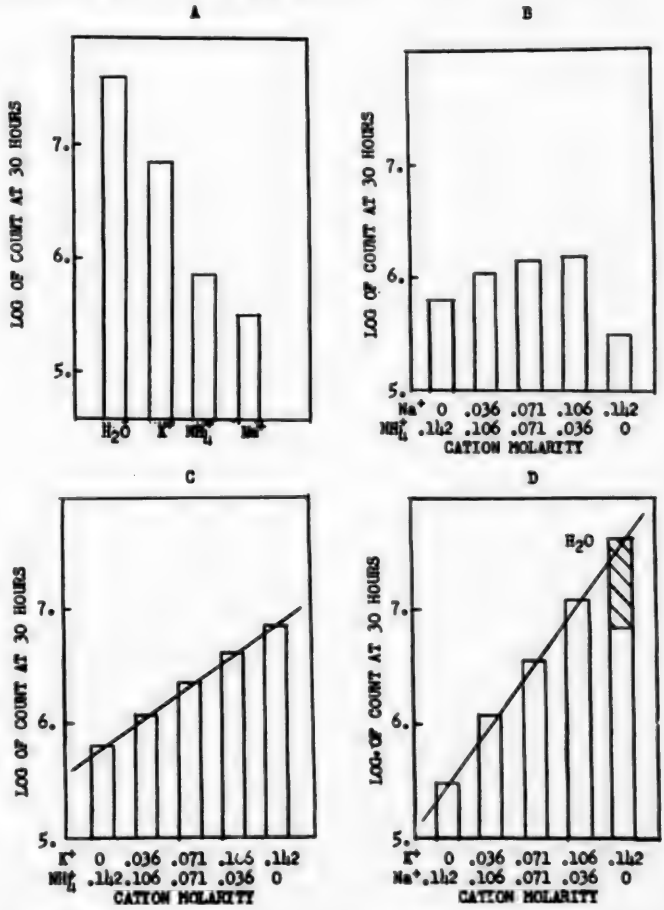

Fig. 4.—(A), (B), (C), and (D). Effect of Na^+, NH_4^+, and K^+ phosphate buffers, pH 7.5 alone and in pairs, upon the growth of *L. acidophilus* 4646 in glucose-nutrient broth. Total cation concentration in all tubes was 0.142 Molar, except in the unbuffered water controls.

Antagonisms between Na^+, NH_4^+, and K^+.—The essential role of potassium in the growth of lactic acid bacteria has been demonstrated by other investigators.[19, 20] Rogosa[19] described a biologic assay for potassium which was based in part upon the finding that sodium and ammonium ions did not replace potassium in the medium that he employed. The test organism was *L. casei 7469*.

MacLeod and Snell,[21] using a K^+-deficient medium which was relatively free of Na^+ and NH_4^+, confirmed that K^+ was essential for the growth of *Lactobacillus arabinosus* 8014, *L. casei* 7469, *Leuconostoc mesenteroides* 8042 and 9135, and *Streptococcus faecalis* 8043. They demonstrated that the magnitude of the K^+ requirement of all of the organisms was greatly increased by the addition of Na^+ or NH_4^+, and they suggested that a competitive relationship between these ions and K^+ appeared to be present. Inhibition of growth was observed when the ratio of Na^+ and NH_4^+ to K^+ concentration exceeded certain limits, and the absolute amount of the ions was not otherwise critical.

Since the medium employed in our investigation supported the growth of *L. acidophilus* 4646, the K^+ concentration was assumed to be adequate. It appeared possible, however, that the addition of large quantities of NH_4^+ and Na^+ might interfere with the utilization of K^+ by this organism in the manner which MacLeod and Snell had described. This was tested by incubating *L. acidophilus* with mixtures of sodium, potassium, and ammonium phosphates in broth at pH 7.6, for 30 hours. It was calculated that in the dilutions used, the 0.38 Molar buffers would yield final cation concentrations of 0.142 Molar.

The results of triplicate counts on each combination are depicted in Fig. 4. As shown in Fig. 4 *A*, the intensity of inhibition at the highest concentration studied was greatest with Na^+, less marked with NH_4^+, and least with K^+. Na^+ and NH_4^+ were the strongest inhibitors, and when each was present in high concentration, growth was poor (Fig. 4 *B*). However, their effects were not additive, since when both were present, each 0.071 Molar, there was less inhibition than when each was used alone at 0.142 Molar. This indicated that Na^+ and NH_4^+ do not exert their inhibitory effect by identical mechanisms, for if that were the case the growth level would be the same for mixtures of these salts in any proportions, provided only that the same molar concentration of phosphate prevailed. Mixture of K^+ with NH_4^+ resulted in release of the inhibition observed with NH_4^+ alone (Fig. 4 *C*). The limit of this release was reached when no added NH_4^+ was present, at which point the inhibitory effect of the potassium phosphate alone was evident.

Mixture of K^+ with Na^+ resulted in release of the inhibition observed with Na^+ alone (Fig. 4 *D*). However, when the ratio K^+/Na^+ was 3:1, growth was better than that obtained with 0.142 M K^+ alone, and the limit of the ability of K^+ to release inhibition of the Na^+ was not the same as for NH_4^+. When this theoretical limit was estimated by extrapolation of the K^+–Na^+ curve, its value corresponded with the observed growth in the water control, in which no added phosphate was present. This further emphasizes the difference between inhibition by NH_4^+ and by Na^+.

All cultures were at pH 7.7 ± 0.1 at the beginning of this experiment. After 30 hours' incubation, the pH of the water control tubes was 6.0 ± 0.1. Most of the buffered tubes did not fall below pH 7.4, but cultures which were only 0.036 Molar (with respect to added cations) did exhibit terminal acidification to as low as pH 7.2.

The foregoing experiment was repeated several times, but exact duplication of the straight-line relationships was obtained with difficulty. They appeared

to be dependent upon a fortuitous combination of proper inoculum and proper time of sampling the cultures. With a view toward controlling these factors, a synthetic medium was sought which would support growth of the organism at pH 7.6, and would give low blank readings in the colorimeter so that growth might be followed turbidimetrically, at convenient intervals.

TABLE IV

COMPOSITION OF DEFINED MEDIUM FOR L. ACIDOPHILUS 4646 (HADLEY)

CONSTITUENT		FINAL CONCENTRATION			
Adenine, guanine, thymine, uracil	each	5.0	microgram/ml	final	broth
L-tryptophan		100	microgram/ml	,,	,,
Glutathione		2.0	microgram/ml	,,	,,
Choline chloride		20	microgram/ml	,,	,,
Inositol		5.0	microgram/ml	,,	,,
L-asparagine		1.0	milligram/ml	,,	,,
L-glutamine		2.0	microgram/ml	,,	,,
Tween 80		0.5	microliter/ml	,,	,,
Calcium pantothenate		1	microgram/ml	,,	,,
Nicotinic acid		5	microgram/ml	,,	,,
p-amino benzoic acid		0.5	microgram/ml	,,	,,
Biotin		0.001	microgram/ml	,,	,,
Folic acid		20	microgram/ml	,,	,,
Riboflavin		1	microgram/ml	,,	,,
Thiamin chloride		2	microgram/ml	,,	,,
Pyridoxine · HCl		0.4	microgram/ml	,,	,,
Pyridoxal · HCl		0.4	microgram/ml	,,	,,
Pyridoxamine · HCl		0.4	microgram/ml	,,	,,
Potassium acetate		6	milligrams/ml	,,	,,
Monopotassium phosphate		0.5	milligram/ml	,,	,,
Dipotassium phosphate		0.5	milligram/ml	,,	,,
Magnesium sulphate (.7 H_2O)		2	milligram/ml	,,	,,
Sodium chloride		0.1	milligram/ml	,,	,,
Manganous sulphate (.4 H_2O)		0.08	milligram/ml	,,	,,
Casein hydrolysate, acid (10%)		0.025	milliliter/ml	,,	,,
D-glucose, anhydrous		10	milligrams/ml	,,	,,
The glucose is autoclaved separately in 10% solution, and is added aseptically to each tube of medium. When this procedure is followed, the addition of					
L-cystein		1	milligram/ml	,,	,,
is made to all of the other constituents, after which this portion of the medium is adjusted to pH 7.0 with 40% NaOH, and autoclaved at 12 lbs. pressure for 15 minutes.					
Stock solutions are preserved with chloroform or toluol, and are stable for about three months.					

Ion Antagonisms in "Synthetic" Broth.—The defined medium of Weisberger and Johnson[22] did not support growth of *L. acidophilus* 4646. The original formula of Landy and Dicken[23] was modified and after considerable experimentation satisfactory results were obtained with the medium shown in Table IV. Marked stimulation of growth was obtained by inclusion of Tween 80.

Preliminary work with the test organism in this medium demonstrated that the progress of growth was most uniform when 2 drops of a 1:10 dilution of a 24-hour culture were inoculated into 5 ml. of the broth. However, when 1 ml. of 0.38 Molar ammonium phosphate buffer (pH 7.4) was incorporated into the medium (final concentration 0.08 Molar), growth did not occur at all if the inocula were diluted beyond that cell concentration. Viable organisms were recovered from the cultures after 6 days, but could not be recovered from cultures inoculated with fewer organisms.

In order to observe the effects of NH_4^+, Na^+, and K^+ upon growth in this medium, the broth was adjusted to pH 7.4, and the respective buffers were added according to the procedure employed in the preceding experiments with nutrient broth. The buffers were of the same concentration (0.38 M), but the pH throughout this experiment was 7.4, instead of 7.6.

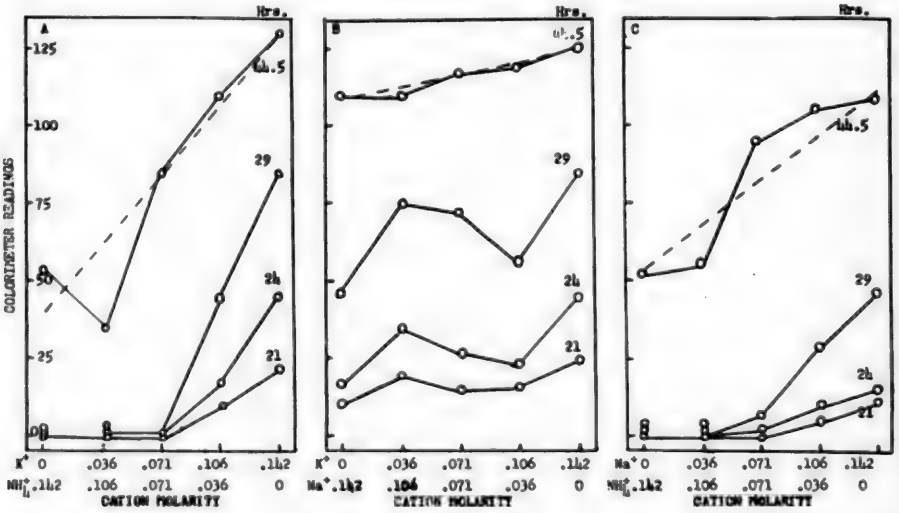

Fig. 5.—Effect of mixed cation phosphate buffers, pH 7.5, on the growth of *L. acidophilus* 4646 in synthetic broth. Readings obtained at the intervals shown.

Sterile colorimeter tubes were used. The tubes contained a final volume of 5.0 ml. and 2 drops of a 1:10 dilution of a 24-hour culture of *L. acidophilus* 4646 were added to each. Readings were obtained with a Klett-Summerson

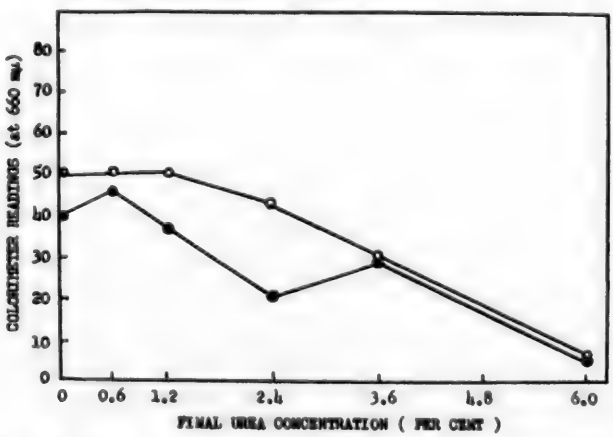

Fig. 6.—The effects of added urea upon growth of *L. acidophilus* 4646 in yeast-extract broth (24 hours' incubation).
●—strain previously trained on 2 per cent urea.
○—original strain.

photoelectric colorimeter, at 660 mμ, after incubation for 21, 24, 29, and 44.5 hours. The findings, shown graphically in Fig. 5, represent the averages of duplicate values.

The same general relationships between NH_4^+ and K^+ were seen. However, the Na^+–K^+ relationship observed with nutrient broth was not operative in the defined medium. Indeed, it appeared that Na^+ replaced K^+ to some extent, with respect to alleviating the inhibition produced by NH_4^+ (Fig. 5 *C*). The time element was an important factor in the linearity of the results.

Effect of Urea Upon Growth.—Preliminary experiments, employing a medium consisting of glucose 1 per cent, yeast extract 0.5 per cent, peptone 0.5 per cent, sodium acetate 1.0 per cent, KH_2PO_4 0.5 per cent, tap water 10 per cent, revealed that growth of the organism was retarded in the presence of 2 per cent urea or more, and that 6 per cent urea prevented the appearance of measurable growth in 24 hours (Fig. 6). A strain which had been subcultured serially in 2 per cent urea showed no greater predilection to grow in the presence of higher concentrations.

The inhibitory effect was demonstrated in the defined medium. *L. acidophilus* was transferred daily in unbuffered synthetic broth, adjusted initially to pH 7.5. Urea was then incorporated into the broth in final concentrations up to 6 per cent (1.0 Molar). The inoculum for the experiments consisted of 2 drops of a 1:10 dilution of a 24-hour culture, added to 5 ml. of the media under investigation. Colorimeter readings at 660 mμ were obtained after 24 hours. The results appear in Fig. 7.

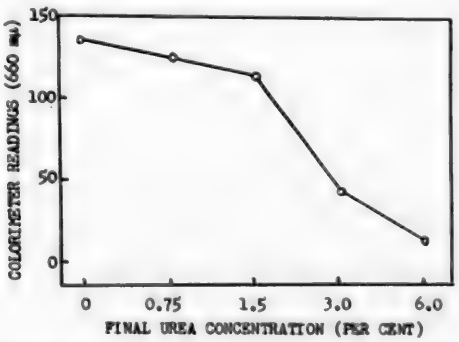

Fig. 7.—Diagram showing growth of *L. acidophilus* 4646 at 24 hours in "synthetic" medium, with different concentrations of urea.

Significant retardation of growth for 24 hours occurred in the tubes containing urea at a concentration of 3 per cent (0.5 Molar), or more.

8. *Effect of Urea Combined With Phosphate.*—Reference has been made to the report that 0.625 per cent ammonium phosphate with 0.375 per cent urea (final concentrations) exerted unusually marked inhibitory influence upon the lactic acid-forming properties of saliva-glucose mixtures, and upon the viability of lactobacilli present in the mixtures initially.[9] The effects were attributed to the specific toxicity of the ammonia that was formed, presumably

by the ureolytic action of other bacteria, during the course of the experiment. In view of the probability that the saliva mixtures contained sufficient K^+ to overcome the inhibitory influence of the amounts of ammonia formed,[24, 25] it was difficult to agree with the foregoing explanation, and the phenomenon was studied with pure cultures of the test organism in the defined medium.

Potassium and ammonium phosphate buffers were prepared at pH 7.5, 0.38 Molar, equivalent to 5 per cent dibasic ammonium phosphate, as described previously. Urea was added in graded amounts to the buffer-broth mixtures, so that its reported potentiating effect upon the magnitude of inhibition could be observed at different concentrations. An unbuffered series of tubes served as controls.

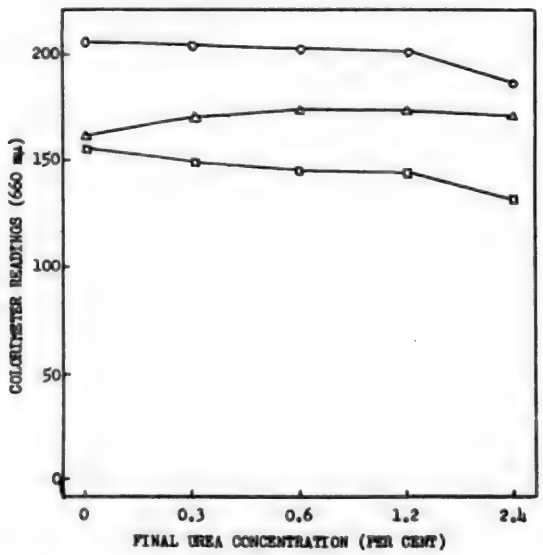

Fig. 8.—Diagram showing the effect of different concentrations of urea on the growth of *L. acidophilus* 4646 in "synthetic" medium, with and without phosphate (24 hours). ○—No phosphate. △—Potassium phosphate. □—Ammonium phosphate. All phosphates 0.08 Molar.

L. acidophilus 4646 was transferred daily in unbuffered synthetic broth, whose initial pH was 7.5. The inoculum for the experiment consisted of 2 drops of a 24-hour culture per 5 ml. of broth-buffer-urea mixture. The experiments were performed in duplicate. After 24 hours' incubation at 37° C., growth was estimated by the colorimeter.

The findings are given in Fig. 8. It is seen that, in the absence of phosphate, urea was not significantly inhibitory even at a final concentration of 2.4 per cent. Inclusion of the phosphates produced the expected inhibition, but the addition of the urea did not make a significant difference, except in the highest concentration, 2.4 per cent, which was more than 6 times as great as that employed by Kesel and his associates.

DISCUSSION

It appears that the inhibitory activity of ammonium is critically dependent upon the composition of the growth medium, the hydrogen ion concentration, the size of the inoculum, and the relative quantities of related ions present in the medium, all of which can be manipulated in vitro, to demonstrate this interesting phenomenon.

The inhibitory action of the urea could not be explained on the basis of osmotic pressure, since equiosmolar solutions of glucose and sucrose were found to exert no similar effect. The buffer capacity of urea was not considered to be an important feature of its inhibitory effect, for, although urea exhibits slight buffer activity below pH 4.0, the experimental cultures seldom approach pH 4 even after one week. The inhibition of growth could not have been caused by ammonia derived from the urea during the course of these experiments, nor by alkalinity due to ammonia formation, because in related studies,[26] it was demonstrated that urea was not hydrolysed by relatively dense suspensions of the test organisms. A more likely explanation of the inhibitory effect of urea would appear to lie in its well-known denaturing action upon proteins.

The foregoing experiments were designed with emphasis upon control of the more conspicuous variables. Efforts were directed toward maintaining relatively constant pH as far as possible during growth. The ionic concentrations of the test compounds were made comparable, at constant pH, except when it was desired to study them at different concentrations. The media employed were nutritionally adequate.

Strict regulation of experimental conditions places definite limitations on the interpretation of the findings. Other investigators have employed techniques which differ somewhat from our own, in certain respects, and have obtained markedly different results. Whereas Kesel, O'Donnell, Kirch and Wach consistently found ammonia to be bactericidal,[7-11] Kirchheimer and Douglas reported failure of ammonium ions to inhibit the growth of oral lactobacilli.[27]

The work of MacLeod and Snell[21] clearly demonstrated the sensitivity of lactic acid bacteria to variation of their ionic environment when that environment is deficient with respect to an essential ion.

It would transcend the scope of this investigation to infer that ammonia may or may not be inhibitory in vivo to lactobacilli in human saliva, whose potassium content[24, 25] and ammonium content[28-30] are such that an effective inhibitory molar ratio of NH_4^+ to K^+ is not likely to be encountered, and whose pH is not usually alkaline, in situ. Similarly, inasmuch as *L. acidophilus* 4646 is only one of many oral lactobacilli, it would be imprudent to draw conclusions relating these findings to dental caries control, particularly while the precise role of lactobacilli in the dental caries process remains a point of controversy.

However, it should be recognized that repeated use of potentially alkaligenic agents in the mouth might eventually result in changing the oral flora toward predominantly nonaciduric forms. Ureolytic bacteria, by liberating ammonia from urea would tend to sustain whatever alkalinity was imparted to the saliva by ammonium phosphate, and acidophilic organisms might be expected to show

a consequent decrease in population. While this bacterial antagonism may be important in the explanation of the observed sporadic variations in salivary lactobacillus counts, it involves little more than the well-known selective influence of hydrogen ion concentration upon the survival or extinction of acidophilic forms in mixed cultures.

SUMMARY AND CONCLUSIONS

1. A study has been made of some effects of ammonium salts and of urea upon the growth of *L. acidophilus* 4646.

2. The growth of *L. acidophilus* 4646 in modified nutrient broth was inhibited by relatively high concentrations of sodium, ammonium or potassium phosphates. The inhibitory effects of ammonium phosphate could be demonstrated only at pH more alkaline than 7.0. Evidence is presented which suggests that other salts are capable of producing similar inhibition provided that they exert buffer effects above pH 7. The action of the inhibitors did not appear to be bactericidal.

3. The effects obtained with inhibitory salts of NH_4^+ and Na^+ appeared to be due in part to the interference of those ions with utilization of the essential potassium ion, and could be partially alleviated by the addition of K^+.

4. A synthetic medium for *L. acidophilus* 4646 is described. While the inhibitory effect of NH_4^+ and the antagonistic relationship between NH_4 and K^+ were further substantiated, it appeared that Na^+ could replace K^+ to some extent, in this medium.

5. The growth of *L. acidophilus* 4646 was inhibited for 24 hours in the presence of 6 per cent urea, and was retarded at lesser concentrations.

6. The growth-inhibiting effects of combinations of urea and ammonium phosphate together in broth were not significantly greater than the effects of each substance when employed alone.

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REFERENCES

1. Bunting, R. W., and Palmerlee, F.: The Role of Bacillus acidophilus in Dental Caries, *J. A. D. A.* **12**: 381, 1925.
2. Hadley, F. P.: Quantitative Method for Estimating Bacillus acidophilus in Saliva, J. D. Res. **13**: 415, 1933.
3. Jay, P.: Lactobacillus acidophilus and Dental Caries, *Am. J. Pub. Health* **28**: 759, 1938.
4. Blayney, J. R., Bradel, S. F., and Hartley, G. F.: Caries Activity as Measured by Quantitative Estimation of Oral Lactobacilli, *J. D. Res.* **18**: 260, 1939.
5. Kesel, R. G.: Dental Caries: Etiology, Control, and Activity Tests, *J. A. D. A.* **30**: 25, 1943.
6. Becks, H., Jensen, A. L., and Millarr, C. B.: Rampant Dental Caries: Prevention and Prognosis, *J. A. D. A.* **31**: 1,189, 1944.
7. Kesel, R. G., O'Donnell, J. F., Kirch, E. R., and Wach, E. C.: The Biological Production and Therapeutic Use of Ammonia in the Oral Cavity in Relation to Dental Caries Prevention, *J. A. D. A.* **33**: 695, 1946.
8. Kesel, R. G., O'Donnell, J. F., Kirch, E. R., and Wach, E. C.: The Amino Acids and Their Deaminating Systems Present in Human Saliva, *Am. J. Orthodont. and Oral Surg.* **33**: 68, 1947.

9. Kesel, R. G., O'Donnell, J. F., Kirch, E. R., and Wach, E. C.: Ammonia Production in the Oral Cavity and the Use of Ammonium Salts for the Control of Dental Caries, *Am. J. Orthodont. and Oral Surg.* **33**: 80, 1947.
10. Kesel, R. G., O'Donnell, J. F., Kirch, E. R., and Wach, E. C.: The Effectiveness of Dentifrices, Mouthwashes, and Ammonia-Urea Compounds in the Control of Dental Caries, *J. D. Res.* **27**: 244, 1948.
11. Kesel, R. G., O'Donnell, J. F., Kirch, E. R., and Wach, E. C.: Recent Developments in the Biologic Production of Ammonia and the Use of Ammonia and Carbamide in Caries Prevention, *Oral Surg., Oral Med. & Oral Path.* **2**: 459, 1949.
12. Stephan, R. M.: Two factors of Possible Importance in Relation to the Etiology and Treatment of Dental Caries and Other Dental Diseases, *Science* **92**: 578, 1940.
13. Stephan, R. M.: The Effect of Urea in Counteracting the influence of Carbohydrates on the pH of Dental Plaques, *J. D. Res.* **22**: 63, 1943.
14. Stephan, R. M., and Miller, B. F.: Effectiveness of Urea and of Synthetic Detergents in Reducing Activity of Human Dental Caries, *Proc. Soc. Exper. Biol. & Med.* **55**: 101, 1944.
15. Muntz, J. A., and Miller B. F.: Factors Influencing Penetration of Synthetic Detergents and Certain Other Compounds Into Dental Plaque Material, *J. D. Res.* **22**: 73, 1943.
16. West, E. S.: Physical Chemistry, New York, 1946, The MacMillan Company, p. 159.
17. Kesel, R. G.: Private communication, December 4, 1948.
18. Weiss, J. E., and Rettger, L. F.: Lactobacillus bifidus, *J. Bact.* **28**: 501, 1934.
19. Rogosa, M.: Microbiological Method for the Quantitative Determination of Small Quantities of Potassium, *J. Biol. Chem.* **154**: 307, 1944.
20. MacLeod, R. A., and Snell, E. E.: Some Mineral Requirements of Lactic Acid Bacteria, *J. Biol. Chem.* **170**: 351, 1947.
21. MacLeod, R. A., and Snell, E. E.: The Effect of Related Ions on the Potassium Requirements of Lactic Acid Bacteria, *J. Biol. Chem.* **176**: 39, 1948.
22. Weisberger, D., and Johnson, F. G.: Nutritional Requirements for an Oral Strain of L. acidophilus, *J. D. Res.* **25**: 35, 1946.
23. Landy, M., and Dicken, D. M.: A Microbiological Assay Method for Six B Vitamins Using Lactobacillus casei and a Medium of Essentially Known Composition, *J. Lab. and Clin. Med.* **27**: 1,086, 1942.
24. White, J., Bunting, R. W., Thomson, K., and Jay, P.: Relation Between Dental Caries and Potassium and Sodium Contents of Saliva, *J. D. Res.* **16**: 315, 1937.
25. Brown, J. B., and Klotz, N. J.: Studies on the Chemistry of Mixed Human Saliva. II. Sodium, Potassium, and Calcium in Salivas Secreted at Widely Varying Rates, *J. D. Res.* **16**: 19, 1937.
26. Pearlman, S.: Influence of Ammonia and of Urea Upon L. acidophilus 4646. II. Studies on Anaerobic Glycolysis. (To be published in *J. D. Res.*)
27. Kirchheimer, W. F., and Douglas, H. C.: The Failure of Ammonium Ions to Inhibit the Growth of Oral Lactobacilli, *J. D. Res.* **29**: 320, 1950.
28. Dodds, E. C.: Cited by Kesel et al, ref. 8, *Brit. J. Exper. Path.* **6**: 266. 1923.
29. Hench, P. F., and Aldrich, M.: Cited by Kesel et al, ref. 8, *J. A. D. A.* **79**: 1409, 1922.
30. Frank, R.: Cited by Kesel et al, ref. 8, *Deutsche Monatschr f. Zahnh* **74**: 567, 1929.

THE USE OF QUATERNARY AMMONIUM COMPOUNDS IN DENTIFRICES

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SINCE the interest in ammoniated dentifrices has opened up a commercial gold mine, there have been numerous attempts to find some other substance which would appeal to the public, cash in on the ammoniated popularity and advertising, and perhaps, incidentally, do as good or perhaps even a better job of caries control. With such an idea in mind, several manufacturers have been working with experimental dentifrices in the hope of producing some such material that would be the panacea for all tooth decay. Interest has, of course, centered on some of the quaternary ammonium compounds following the reports in the literature of the bacteriocidal, detergent action, and general usefulness of these compounds.

Quaternary ammonium compounds as bacteriocidal agents first came into prominence in 1935 when Domagk[3] reported his findings in the investigation of a series of quaternary ammonium compounds. Previous work had been done by others[5, 6] but the potential of this class of compounds was not exploited until Domagk awakened the research workers' interest in these versatile and highly effective germicides. Since that time the literature has grown tremendously.

The application of these compounds to dentifrices was a natural outgrowth and the logical conclusion of utilizing the effect of a surface lowering agent was evident inasmuch as the penetration of the various organisms would of necessity be intensified. For example: Grace and Bryson[1, 2] have shown that the use of detergents in various infections, when combined with one of the antibiotics reduces bacterial counts and the duration of the diseases. Miller and his co-workers[11, 12] investigated the effect of quaternary compounds on the organisms associated with dental caries, and Huyck[7] studied the effect of one of the quaternaries on the oral flora. We believe, however, that this is the first time that there has been an attempt to evaluate the use of quaternary compounds in clinical practice when they are used in the form of dentifrices.

PROPERTIES

The quaternary ammonium preparations are compounds that are nucleated about a 5-co-valent nitrogen, with 4 of the valences satisfied by organic radicals, the fifth is usually satisfied by a halogen—usually chlorine or bromine. There are a great many different combinations of these compounds available comercially under a variety of identifying trade names. The variations occurring are generally in the nature of organic substitutions. The nature of these organic substitutions is of a great deal of importance in the combining power and practical application of the quaternary to the vehicle in which it is used.

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The properties of this group of compounds is unique for they possess surface activity, bacteriocidal or bacteriostatic activity, and in some cases, detergency. Not all quaternaries possess all three of these properties, but they may or may not possess any combination of the three. These 3 properties may be individually or severally affected and influenced either synergistically or antagonistically by the nature of the chemical or physical environment with which the compound may come in contact. This fact of adverse environmental effect presents one of the major problems in the formulation, stability and germicidal activity of a dentifrice containing a quarternary ammonium compound. Quaternary ammonium compounds are generally stable against most reagents; however, they may be completely unstable in the presence of organic materials. In the mouth, the presence of blood, pus, food debris and other materials may affect the efficiency of the compound. They frequently take part in double decomposition reactions to form salts; they appear to be resistant to boiling, being thermally decomposed only at high temperatures; decomposition occurs more readily in strong alkalies.[4] Usually these alkali concentrations are much higher than would or could be used in dentifrices or even in most medicaments.

The surface activity of the quaternary compounds contributes markedly to the oxidation of metals. This characteristic makes the selection of preparation equipment important, and the proper type of stainless steel (preferably Monel) should be selected for processing, mixing, and preparation equipment. This fact also makes the selection of suitable containers for storage and distribution an extremely important item from a technical as well as an economic point of view.

COMPATIBILITIES AND INCOMPATIBILITIES

Compatibilities and incompatibilities coupled with the property of entering into double decomposition reactions are of major concern. Most quaternaries are capable of being rendered bacteriocidally inactive by many of the common and usual ingredients put into dentifrices. The reason for this is that the quaternaries are cation-active, and are precipitated by anion-active substances such as soaps or synthetic soaps such as the sulfonated fatty acids. This would of necessity preclude the use of the commonly employed detergents in a dentifrice containing a quaternary compound. Those detergents, however, which are nonionic function well with the quaternaries, and with care in selection, a good foaming detergency can be attained. It should be pointed out that some nonionic compounds function as cations at a low pH, but as anions when the pH is raised to a high level. Therefore, even the selection of compounds of this type should be thoroughly tried before being incorporated into a nonionic compound in a dental formula. While foaming is not essential as a characteristic of a dentifrice, it has been found to have marked consumer appeal and, therefore, has done much commercially to induce consumer acceptance of one product over another.

We have found that the selection of a nonionic type of a detergent may produce unexpected and unpleasant results. For example, the use of certain quaternary compounds and nonionics cause the deposition of an undesirable film on the teeth surfaces; most of this film causes the adherence of staining material. This leaves a marked discoloration and is unpleasant and unsightly. Not much is known concerning the nature of this film deposition although it is not new in the use of quaternary compounds in other nonrelated fields. It has been established in work with sanitizing solutions employing mixtures of a quaternary and a nonionic, that the presence of protein in the form of milk or tanninlike substances such as are found in coffee, leave a tenacious film that is removed with great difficulty from the surfaces of utensils. Quaternary compounds are endowed with the ability to leave surface films; what the nature of this film is and what role it plays is a matter of several conjectures and some theorizing. It leaves a film on smooth surfaces in particular and on any surface in general.[10] The film so deposited on teeth is of the same general type and evidence as to its nature has not been revealed. The general appearance is a marked discoloration which leaves a stain shading from light yellow to orange to a dark brown. This cannot be removed except by complete and thorough prophylaxis. It is possible that it is due to the pH of the mouth or the pH of the saliva as freshly secreted; to the presence of organic materials from food or tissue proteolysis. From other experiments, it has been shown that removal of any one factor, such as the organic radical or environmental factors, change in the quaternary or the nonionic material, would prevent the formation of stain.[8] In view of these variations, further investigations of the use of various detergent mixtures are under way in our laboratory. We feel that film-forming properties are desirable when the film is pure quaternary or even an active salt of a quaternary because it leaves a protective factor upon areas and locations vulnerable to caries attack; this may be a mechanical protection, but there may be as well a cumulative action of the bacteriocidal activity of the quaternary film by itself.

Incompatibilities of the quaternaries limit the use of many conventional dentifrice ingredients, and preclude the use of essential oils and the saccharins; for example: oil of peppermint is unstable. Menthol may be used to supplant the peppermint or an oil such as clove and spearmint seem to be stable. These may make the dental formula palatable. Some of these oils may mask the bitter taste of the quaternary without the addition of other sweetening substances. Traces of amino-acetic acid may be used and are considered to be beneficial.

Organic binders interfere with the action of the quaternary with the exception of the glycerols and perhaps the simple ethers of cellulose. Alginates being destructive to the quaternary molecule are decidedly incompatible and are to be avoided.

CHEMICALLY TESTING THE DENTIFRICES

Testing the compatibility of various ingredients should be done chemically; the mixture of the quaternary and other ingredient should be done

in proper concentration and should be observed for precipitation and clouding. Not all incompatible mixtures yield these phenomenon. Bacteriocidal tests should be done for active formulation.

Quantitative control of the quaternary content should be well regulated, each batch being tested to assure proper concentration. Many methods are reported in the literature, but the Kjeldahl nitrogen determination proves the most accurate. Analysis by the DuPont nitrometer method has value if the collected gas is properly washed to remove the carbon dioxide resulting from the acid decomposition of the precipitated calcium carbonate. This latter method has not been sufficiently investigated.

The toxicity data for quaternary ammonium compounds indicate that they are nontoxic in concentrations known to be germicidally effective.[9] Most of these data have been collected by the various manufacturers of the compounds. The toxicity studies on the finished dentifrices show them to be of the same type of nontoxic products when used daily for a period of 2 years or longer.

BACTERIOLOGICALLY TESTING THE DENTIFRICES

The bacteriocidal activity of a dentifrice is of great importance and whether one subscribes to the theories of the mechanical cleansing action of tooth brushing, to the liberation of ammonia, or to the action of an antiseptic is immaterial. The bacteriological methods for testing are becoming a routine procedure and while there are numerous variations in technique, the purpose of evaluating the lethal action of the dentifrice on test organisms is the prime consideration.

Organic matter materially reduces the bacteriocidal activity of most of the quaternaries.[13-18] There are a few instances where the presence of horse serum enhanced the bacteriocidal action. It has been demonstrated that the presence of certain electrolytes with quaternary compounds enhances the bacteriocidal activity of the compound. In horse serum there is frequently a concentration of electrolytes which may overcome the usual inactivation.

The status of the bacteriological tests for quaternary compounds is, at present, in a state of confusion. This is due to the fact that the old standards of phenol coefficient methods do not hold for these bacteriocides. Reddish[19] gives a review of most of the methods and we have found that the method of Klarmann and Wright[20] is the most suitable.

The technique developed for the testing of quaternary dentifrices in this laboratory has been made as simple as possible and is as follows: *Materials:*—Sterile small and large whiskey glasses with paper covers, sterile glass rods, sterile wooden applicator sticks, wire loop No. 5, sterile saline, 24-hour culture of *Lactobacillus casei*. *Procedures:*—(1) A sterile whiskey glass is placed on each pan of a Torsion balance and balanced. (2) One gram of tooth paste is placed into the sterile whiskey glass using a sterile applicator. The sterile paper cover is replaced. (3) One gram of tooth paste is weighed into three other glasses in the same manner. 0.25 Gm. of tooth paste is weighed in one

TABLE I

STOCK TOOTH PASTE
SALINE DILUENT

ORGANISM	1:3	1:6	1:12	1:24	1:100	CONTROL	EXPERIMENT TIME (MIN.)
Lactobacillus casei—Hadley	+	+	+	+	+	+	1
	+	+	+	+	+	+	3
Lactobacillus oral strain	+	+	+	+	+	+	1
	+	+	+	+	+	+	3
Aciduric streptococcus oral strain	0	+	+	+	+	+	1
	0	+	+	+	+	+	3

0 = no growth.
+ = growth.

glass. 1-100. (4) Dilutions are made according to the following scheme: 1:3—1 Gm. paste, 1 c.c. saline, 1 c.c. culture; 1:6—1 Gm. paste, 2.5 c.c. saline, 2.5 c.c. culture; 1:12—1 Gm. paste, 5.5 c.c. saline, 5.5 c.c. culture; 1:24—1 Gm. paste, 11.5 c.c. saline, 11.5 c.c. culture; 1:100—0.25 Gm. paste, 12.4 c.c. saline, 12.4 c.c. culture. One dilution is made at a time. (5) The paste, saline and culture are mixed together with a sterile glass rod. After 1 minute a sub-culture is inoculated with a No. 5 loop into Difco Thioglycolate broth. The mixing is continued and a second sub-culture made after 3 minutes into the same medium. (6) Cultures are made and read 24 and 48 hours after incubation at 37.5° C. For the in vitro testing of dentifrices following the previously outlined techniques, standard strains of bacteria were tested against the various dentifrices. The results are shown in Tables I and II.

CLINICAL EVALUATION OF DENTIFRICES

Dental and oral hygiene students were taken as subjects for clinical evaluation so that as many factors as possible could be controlled. In addition, the social and economic levels, the mouth hygiene, and health of all students was grossly within the same range as determined by a member of the dental faculty. Gross discrepancies were eliminated. One hundred students were used in each group and the study carried over a 2-year period.

Additional bacteriological techniques were used to study the effect of the dentifrices on the total bacterial count as well as on the lactobacillus count.

TABLE II

QUATERNARY AMMONIUM TOOTH PASTE
SALINE DILUENT

ORGANISM	1:3	1:6	1:12	1:24	1:100	CONTROL	EXPERIMENT TIME (MIN.)
Lactobacillus casei—Hadley	0	0	0	+	+	+	1
	0	0	0	+	+	+	3
Lactobacillus oral strain	0	0	0	+	+	+	1
	0	0	0	0	+	+	3
Aciduric streptococcus oral strain	0	0	0	+	+	+	1
	0	0	0	0	+	+	3

0 = no growth.
+ = growth.

TABLE III

COMPARATIVE BACTERIOLOGICAL STUDY
TOTAL BACTERIAL COUNT OF 100 CASES

PRODUCT	PRETEST	5 MIN.	30 MIN.	3 HRS.
Stock paste	68	30	41	118
% reduction		56	40	88% increase
Hyamine 10X	86	19	20	43
% reduction		76	77	50% decrease
Hyamine 1622 paste	77	34	31	26
% reduction		56	59	63% decrease
Precipitated chalk alone	90	74	56	120
% reduction		18	39	33% increase

Average in millions per c.c.

For this technique, the standard methods of mouth rinsing were used. Plating and all dilutions of the rinses were made on tomato juice agar, Snyder's agar and F.D. agar. Mouth rinses were made pretest followed by the subject brushing his teeth in a supervised technique for 2 minutes. Following this, rinses were again made at 5-minute, ½-hour and 3-hour intervals. Average counts were then made on all types of test agar. Results obtained are listed in Table III.

A second group of students were studied to determine the effect of the various types of paste on the lactobacilli; both smooth and rough colonies were counted using the general bacteriological techniques and media described above. Four pretest counts were made to establish a normal average. Weekly counts were collected at the same hour of the day and one hour following brushing with the agent.

COMMENTS

The clinical testing of quaternary ammonium compounds has shown that they are inhibitive toward the general oral flora, but not to the lactobacillus group in particular. This is in part due to the organic material in the saliva and to other glandular and cellular secretions which are always present within the mouth and perhaps due to organic decomposition materials of foods of various types. The future study of these compounds in dentifrices must deal with combinations of ingredients whose synergistic action will overcome the inactivating effect of organic contaminants found in the oral cavity. Much work is yet to be done on this form of dentifrice. One study under investigation is the correlation over a long period between the use of a quaternary and other dentifrices with mouth roentgenograms to compare caries activity.

TABLE IV

LACTOBACILLUS COUNTS
AVERAGES 100 CASES

PRODUCTS	PRETEST COUNTS	POST-TEST COUNTS	% OF CHANGE
Stock paste	39,000	21,000	46 reduction
Hyamine 1622	159,000	262,500	44 increase
Hyamine 10X	76,000	44,000	59 reduction
Ammoniated not quaternary	210,000	146,000	30 reduction

REFERENCES

1. Grace and Bryson: *N. E. Jour. Med.* **237**: 683, 1947.
2. Grace and Bryson: *Surg., Gynec. & Obst.* **91**: 333, 1950.
3. Domagk: *Deutsch. Med. Wchnschr.* **61**: 829, 1935.
4. Corin: *Rev. Geol.* **13**: 4.
5. Jacobs and others: *Jour. Exper. Med.* **23**: 569, 1916.
6. Jacobs and others: *Jour. Exper. Med.* **23**: 577, 1916.
7. Huyck: *Jour. Am. Pharm. Assoc.* **34**: 5, 1945.
8. Personal communication.
9. Shelanski: *Soap & San. Chem.* **25**: 125, 1949.
10. Miller, Abrams, Huber, and Klein: *Proc. Soc. Exper. Biol. & Med.* **54**: 174, 1943.
11. Miller, Baker, and Harrison: *Proc. Soc. Exper. Biol. & Med.* **42**: 705, 1939.
12. Miller, Muntz, and Bradel: *Proc. Soc. Exper. Biol. & Med.* **45**: 104, 1940.
13. Johns: *Can. J. Res.* **26F**: 91, 1948.
14. Van Eseltine, and others: Tech. Bull. No. 282, NYS Agr. Exp. Sta. April, 1948.
15 Lawrence: *J. Am. Pharm. Assoc. Sci. Ed.* **37**: 57, 1948.
16. Lawrence, Kwartler, Wilson, and Kivela: *J. Am. Pharm. A.* (Scient. Ed.) **36**: 353, 1947.
17. Mallmann, Leavitt, and Joslyn: *Proc. Soc. Exper. Biol. & Med.* **68**: 401, 1948.
18. Quisno, et al.: *Am. J. Pharm. A.* (Scient. Ed.) **35**: 317, 1946.
19. Reddish: *Drug & Allied Ind.*, Feb., 1950, pg. 9.
20. Klarmann, Wright, and Shternov: *Am. J. Pharm.* **122**: 1, 1950.

DENTINAL PROTEIN

Recovery in Pure Form

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THE amount of dentinal protein in human teeth has been reported in numerous papers. Leicester[1] pointed out that the significant disparities in results are probably due to the different manner in which each investigator has handled the samples with which he has worked.

This investigation was undertaken to determine what modifications of sample preparation and dialysis would produce the highest yield of dentinal protein, and at the same time account for the greatest percentage of the total amount of nitrogen known to be present in the dentin. The results of this investigation are the subject of this paper.

In the past, Bowes and Murray,[2] Armstrong and Brekhus,[3] and Armstrong,[4] as cited by Leicester[1] have proposed values for dentinal protein content ranging from 21.00 to 22.11 per cent, based on nitrogen analyses. This method assumes one of two sets of conditions: (a) that the percentage of nitrogen in each compound is known, and the percentage of each compound in the dentin is also known; or, (b) that all the nitrogen is contained in protein, and that the percentage of nitrogen in the protein is known. However, Pincus[5] demonstrated the presence of at least one nonprotein nitrogenous compound from dentin which was neither isolated nor known to be present at the time the calculations mentioned were made. For this reason alone, percentages of protein arrived at through calculations based on nitrogen are, in part, suppositions.

LeFevre and Manly[6] assign a protein content value of 17.5 per cent to dentin by difference, after all other constituents are known. This is an ever-changing figure, diminishing whenever an additional constituent is reported, or changing whenever the percentage of a known compound is better determined.

Hess, Losee, and Neidig[7] used a dialytic method for the isolation of dentinal protein, similar to that for work with enamel protein. Their yields are very low, a result probably caused by the solubility of protein and also by slow digestion of the protein during the long dialysis time used.

METHODS AND CONSIDERATIONS

Factors held constant throughout this investigation were: techniques of sampling, preparation and drying of dentin samples, demineralization, nitrogen analysis, and ashing. Particle size distribution varied through small limits. Variables were: acid concentration, stirring, temperature, and time of demineralization.

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Sampling.—One of the more important explanations of the wide range of analytic values reported for human teeth is the almost complete lack of uniformity of sampling. Following is the procedure developed for this study, together with some of the justifications for the technics involved.

Teeth, each from the same location in the arch, were placed in groups of from four to ten, and each group was used in the preparation of a separate sample. Upper and lower teeth were analyzed separately. Teeth selected were free of caries, stain, hypoplasia, mottling, and abnormal morphology and color. The apices were closed except in one sample noted in Table II.

We have access to teeth from service personnel of a rather constant age group. In order to use the least affected teeth in the mouth, it was decided to use only crowns from erupted third molars, taken from normal, healthy men 18 to 23 years of age. Since the third molar is mineralized twelve years after birth,[8] the crowns used in this study had been in the mouth one to six years and had been calcified six to eleven years. Only the crowns were used, for the purpose of preparing dentinal protein from that part of the tooth most often attacked by caries and for the added reason that root dentin may or may not have the same analytic behavior and values.

Isolation of Coronal Dentin.—To secure coronal, primary dentin in as near its natural state as possible, the following procedure was used.

(1) On extraction (with elevators—no forceps used) the teeth were placed immediately in a paper cup and this was put into a freezing unit. Once a day these were collected and placed in test tubes with two drops of water, stoppered with rubber stoppers, and placed in a freezer kept at –20° C. This kept the teeth frozen and hydrated until ready for use.

(2) When a tooth was to be used it was removed from the freezer and scrubbed clean with a surgeon's Nylon brush. After cleaning, the crown was severed from the tooth about 1½ to 2 mm. above the cemento-enamel junction. This precluded the inclusion of cementum, pulp, or secondary dentin in the sample. The cutting was done with a safe-sided diamond disk at low dental engine speed. The metal disk with diamond was selected because it generates the least heat in the tooth during cutting.

(3) The crown was then embedded (dentin up and exposed) in plaster in a Petri dish, to give lateral support to the enamel walls; pink plaster was used to give contrast. Just prior to the initial set of the plaster a few drops of water were added to the Petri dish; it was then covered and returned to the freezer.

(4) After an hour in the freezer the plaster block was removed and the dentin ground out of the crown with a size 8 or 9 carbide-steel round bur, rotating at the slowest speed on a standard dental engine. Minimum pressure was used throughout the cutting process. As grindings collected they were tapped into a Petri dish and kept covered. On nearing enamel, burs of regular steel and smaller size were used to permit removal of dentinal cusps. The use of slow speed, minimal pressure, and regular steel burs reduced to a minimum the possibility of contamination of the dentin by enamel. Fifty to sixty minutes are required to bur the dentin from each crown.

(5) As soon as a group of crowns was completed, the grindings were transferred to a ground-glass stoppered weighing bottle and weighed. The stopper was then removed and placed with the bottle in a desiccator. The desiccant used was fresh, 8-mesh $CaCl_2$. The sample was allowed to remain in the desiccator until constant weight was reached—five to six weeks.

Our choice of freezing as a method of preserving whole teeth may be open to criticism. However, freezing and preserving at low temperatures is to our minds the least destructive method of preservation. If Formalin is used, there is definite change in protein—tanning. Experiments are under way now to determine the effect of this tanning on the yield of protein obtainable by demineralization. Preservation in alcohol and in chloroform is also being investigated from the same standpoint.

Considerations on Drying Dentin.—In drying dentin the following characteristics of technique and results are desirable: (1) reproducibility, (2) least amount of change and no loss of nitrogen, and (3) lowest solubility of the resulting dentin.

To determine drying behavior, samples were subjected to a variety of tests. Small samples were found to turn yellow at temperatures around 160° C. It was suspected that there was loss of nitrogen also at such temperatures. To verify this suspicion, a large sample which had been air dried a few days was weighed and treated as indicated in Table I. It is evident that nitrogen is lost if dentin is heated to temperatures around 160° C.

TABLE I

EFFECT OF TEMPERATURE ON DRYING AND NITROGEN CONTENT OF DENTIN

TIME	CONDITIONS	TOTAL WT. LOST	CUMULATIVE WT. LOSS (%)	PER CENT NITROGEN
1 day	Room temp. over $CaCl_2$	1.73	----	3.1
10 days	Room temp. over $CaCl_2$	1.00	2.73	----
4 hr.	37.5° C., open air oven	—*	----	----
4½ days	75° C., open air oven	2.27	5.00	3.16
1 day	97° C., open air oven	1.68	6.68	----
1 day	111° C. over $CaCl_2$	0.55	7.23	3.28
1 day	111° C. over $CaCl_2$ at pressure 1 mm. Hg	0.60	7.83	3.33
1 hr.	164° C., open air oven	0.52	8.35	3.21
1 day	164° C., open air oven	0.58	8.93	3.02

*Gained weight during the four hours; three one-hour repeat tests were made, and the sample gained weight each time.

Two other large samples were placed over $CaCl_2$ at room temperature within one hour of the finish of grinding and weighing. In thirty-four days these samples attained constant weight. All weighings were made with stoppers in bottles, as dentin becomes quite hygroscopic after it is partially dehydrated. These two samples lost 6.48 per cent and 6.51 per cent in weight respectively during the thirty-four days required for their drying, and remained constant in weight thereafter.

A third experiment consisted of drying a large sample to constant weight (forty days) over $CaCl_2$ at room temperature and then splitting it into three portions. Although we felt that temperatures around 100° C. would produce

a constant-weight sample of dentin, earlier experiments had indicated that the dentin so dried was more easily hydrolyzed or dissolved. Also, it was necessary to know how much nitrogen could be lost from a sample in the 160° C. range, and whether such a sample would reach a constant weight and nitrogen content or not.

The three portions of this latter sample were treated as follows.

Portion A: Remained over $CaCl_2$ at room temperature and remained constant in weight for thirty-eight days more, at which time it was removed and demineralized. Nitrogen content of the dentin remained constant at 3.2 per cent.

Portion B: Was placed in an open air oven at 108 to 110° C. and after further weight loss of 2.3 per cent it reached constant weight in forty-one days. It then remained constant for eight days longer, at the end of which time it was removed and demineralized. The nitrogen content was raised to 3.3 per cent by the added water loss.

Portion C: Was placed in an open air oven at 155 to 160° C. and found to lose weight over a period of eighty days, never becoming constant. In this time it lost 11.1 per cent of its weight and became a dark brown in color. It was removed and demineralized, its nitrogen content having dropped to 1.0 per cent.

The results of the demineralizations were:

A: 9.11 per cent yield of protein (four days' demineralization).
B: 6.24 per cent yield of protein (two days' demineralization).
C: 0.46 per cent yield of material (two days' demineralization).

As expected, the heating of B had produced a sample more soluble during demineralization. What nitrogen there was in C was all recovered in soluble form at the end of the demineralization. The solid residue from C's demineralization was dark brown and appeared charred; it was such a small amount that nothing was done with it.

These results led to the decision to use room temperature desiccated dentin. From five to six weeks are required to attain constant-weight samples by this method. During the latter part of the drying, the rate of weight loss becomes quite small—of the order of 0.05 mg. per day. For this reason a sample is not judged dry until it has been constant in weight for at least a week.

Particle Sizes of Ground Dentin.—Ground samples of dentin were placed in a standard sieve set and shaken 15 minutes. Approximately 50 per cent of the sample passed 140 mesh, and less than 10 per cent failed to pass 60 mesh. No part of the sample was discarded because of particle size. A study of the effect of particle size on analysis and dialysis is being conducted.

Demineralization.—A specially shielded, Pyrex adapter (Eimer and Amend No. 1-032) was used as a dialysis chamber. This apparatus possesses many advantages over other commonly used chambers; it is easy to handle and presents a large surface over which the sample spreads easily; it allows gas formed to escape freely, yet remains dust free; it works well with a stirred solution, permits easy removal of salts formed by gravity, and, finally, it gives a secure purchase for tying in place the sheet Cellophane used as a dialysis membrane.

The membrane is placed before the introduction of the sample. This membrane consists of two sheets of nonwaterproof Cellophane, previously wet with distilled water, tied over the large mouth of the adapter with three separate ties of regenerated cellulose fiber.*

Weighed samples of the dentin are placed in the small neck of the adapter and washed onto the inner surface of the membrane with a portion of the demineralizing fluid. This insures that all the sample is on the membrane. The total volume of the demineralizing fluid used in our early work was 1 liter—50 ml. in the adapter and 950 ml. in a beaker. Later we found that 1 ml. of acid per milligram of sample was sufficient, and that the ratio of inner fluid to outer fluid should be 1:15, approximately.

After the sample and the inner fluid are in the adapter, the remainder of the acid is placed in a 1 liter beaker and the adapter is clamped in such a manner that the inner and outer fluid levels are the same. A piece of rubber-dam is placed over the neck of the adapter so as to cover the beaker, and the small end of the adapter is plugged loosely with a notched cork or cotton to prevent the entrance of dust. A thermometer and, when used, a stirrer are introduced through the rubber-dam. As a check on the amount of nitrogen introduced by this procedure, blank runs were made from time to time using the complete apparatus, omitting only the dentin sample. The inner and outer solutions of the blank were then analyzed for nitrogen after allowing it to stand the same time as the samples stood.

On stopping demineralization, three fractions—inner fluid (IF), outer fluid (OF), and insoluble protein—are present to be separated, sampled, and analyzed. The adapter and its contents are first removed to another beaker; the volume of outer fluid remaining is measured and aliquot samples taken. The Cellophane is then cut from the adapter and the inner fluid and insoluble protein are washed into the clean beaker. From this beaker the slurry is then washed into large centrifuge tubes. The total volume of wash water used is recorded.

The centrifuge tubes containing the inner fluid and protein are spun at 3,000 r.p.m. for 15 to 20 minutes. The inner fluid is then decanted into a sintered glass funnel (fine pore size) and filtered under suction at a water pump. The suction is broken as necessary to prevent frothing. The inner fluid volume is measured and aliquots are taken for analysis.

The insoluble protein is washed, in the centrifuge tubes, successively with 50 per cent ethanol, absolute ethanol, 50 per cent ether in absolute ethanol, and, finally, twice with ether. Each washing consists of thoroughly stirring the protein in the wash liquid, centrifuging 10 to 15 minutes at 3,000 r.p.m., and decanting the wash. All washings are collected in the same flask, reduced in volume under diminished pressure, and aliquots are analyzed for nitrogen.

The insoluble protein from the final ether wash is desiccated one to two days in the centrifuge tube, transferred to a tared weighing bottle, and, finally, dried to constant weight over $CaCl_2$ at room temperature.

*Cellophane from Wm. Dixon, Inc., No. 11-645; cellulose fiber from L. D. Caulk Co., No. M1064.

Nitrogen Analysis.—

Size of sample used:

Dentin	15 to 25 mg.
Protein	8 to 15 mg.
Inner fluid	10 to 15 ml.
Outer fluid	15 to 20 ml.

Digestions are carried out in a Clough nitrogen apparatus, using 25 ml. Pyrex Kjeldahl flasks. A selenized granule is used as a catalyst and to prevent bumping. After the digestion mixture is clear the digestion is carried on for one hour; shorter digestion after clearning produces erratic results, longer periods do not give any higher results and add the danger of a flask going to dryness.

Distillation is carried out in an improved Kemmerer-Hallett Pyrex nitrogen apparatus. This ran more smoothly and satisfactorily after the glass condenser had been cut off and a water-jacketed silver tube was substituted in its place. Distillations are carried out with the tube under the surface of the collecting fluid 4 minutes and over the surface 1 minute. The collecting fluid is 2 per cent boric acid which contains 16 ml. of mixed indicator (prepared according to Ma and Zuazaga[9]) per liter.

Following the distillation, the solution is titrated, using 0.01N HCl. If the volume of acid required is small, a platinum-tipped microburette is used.

Ash Determinations.—

Size of sample used:

Dentin	10 to 20 mg.
Protein	5 to 12 mg.

Ashings are carried out in a small platinum boat shielded by platinum foil. An electric, porcelain baking oven, equipped with a thermocouple, is used. Results obtained are comparable to careful incinerations made in platinum crucibles over Fisher burners.

The boat bearing the sample is placed in the cold furnace and the door left open ⅛ inch. The furnace is turned on at the lowest heat and in one hour the temperature of the oven is 150° C. This temperature is maintained for two successive hours; the door is then closed and the temperature rises to 1,100 to 1,150° C. over a period of another hour. The high temperature is maintained for two hours, and the oven is turned off. When the furnace cools to 100° C. the boat is removed and cooled to room temperature in a desiccator. It is then weighed.

The advantage of the foregoing procedure lies in the fact that the residue produced does not change (a) by treatment with concentrated HNO_3, (b) by further heating, (c) by cooling in a desiccator from maximum temperature, or (d) on standing open to the atmosphere on the balance pan.

RESULTS

Effect of Acid Concentration on Demineralization.—These experiments were designed to show the effect of acid concentration on the yield of insoluble pro-

TABLE II

RECOVERABLE PROTEIN OF HUMAN TOOTH DENTIN

		NUMBER OF SAMPLE						
		I	II	III	IV	V	VI	VII
1	Identity of teeth in sample	17 and 32	17 and 32	17 and 32	1 and 16	17 and 32	17 and 32	17 and 32
2	Number of crowns in sample Roots open (O) or closed (C)	7(C)	6(C)	7(C)	7(C)	6(C)	4(O)	7(C)
3	Milligrams of dentin demineralized	555	660	460	960	465	750	724
4	Per cent ash loss of original dentin	28.5	27.9	28.5	29.4	27.9	29.7	28.5
5	Per cent nitrogen in original dentin (0.10%)	3.28	3.27	3.28	3.30	3.27	3.30	3.28
6a	pH of demineralizing HCl Initial	2.9	1.95	1.80	1.03	1.03	0.73	0.73
b	Final	6.3	3.3	2.24	1.08	1.10	0.75	0.75
7a	Volume of HCl used Inner fluid	50	50	50	55	40	50	50
b	Outer fluid	950	610	950	905	425	950	950
8a	Time of demineralization in days	3	2	3	2	2	3	3
b	Stirred (S) or not stirred (N)	S	S	S	N	S	N	S
c	Mean temp. of demineralization	24° C.	28° C.	24° C.	21° C.	23° C.	23° C.	29° C.
9	Per cent of original dentin recovered as insoluble protein	75.7	19.58	14.65	15.65	15.10	15.20	11.97
10	Per cent ash residue of insoluble material	71.5	17.64	1.02	0.60	0.60	0.60	0.60
11	Per cent nitrogen of insoluble material (0.3%)	3.55	13.65	16.04	16.24	16.10	16.11	16.41
12	Per cent nitrogen of original sample weight in:							
a	Insoluble material	2.69	2.67	2.35	2.54	2.45	2.45	2.00
b	Inner fluid	0.11	0.31	0.34	0.47	0.49	0.57	0.93
c	Outer fluid	0.15	0.12	0.34	0.17	0.17	0.21	0.15
d	Rinses (ether removed under vacuum)	Not run	0.02	Not run	0.06	0.07	0.01	Not run
13	Sum of lines 12 a, b, c, and d	2.95	3.12	3.03	3.24	3.18	3.24	3.08
14	Per cent of original nitrogen accounted for (line 13 divided by line 5)	89.9	94.8	92.3	98.1	97.2	98.2	93.9

tein recovered from the acid dialysis of ground dentin. Our twofold objective was to account for all the nitrogen in the original dentin and to secure as much pure protein as possible.

Acid concentrations used are designated in pH units. Using a Beckman Model G meter as a measuring device for pH, HCl of varying pH values was made from constant boiling acid.

Table II gives an all-inclusive picture of some representative experiments. The effect of acid concentration is shown as it relates to the inner and outer fluids of the dialysis, as well as to the yield of protein. The completeness of demineralization is also shown.

Total nitrogen recoveries of the order of 98 per cent are obtained by using HCl in the pH range 1.0 to 0.7. These recoveries are obtained in forty-eight hours, without the aid of stirring, and at normal room temperatures. When the losses of material inherent to the procedure are considered, it is clear that all the nitrogen has been accounted for.

The yield of protein recovered is approximately 15.5 per cent of the weight of the original dentin sample. This is considerably lower than the predicted values in the literature. These reported values, it should be noted, are probably the predictable maximum, while our values more nearly approach the recoverable maximum, with the methods described.

The nitrogen percentage of the original sample found in the outer fluid is of rather constant value. This would indicate: (1) that a certain amount of deamination takes place under any condition, without respect to time, or (2) that small amino acid residues are produced equally in all of our experiments on hydrolysis, or (3) that there are some nitrogen compounds present in fairly constant amounts which are crystalline, or at least small enough to pass the membrane used. There is a fourth possibility, namely, that the membrane pore size reduces to the point that no more nitrogen compounds may cross through after a certain time or concentration is reached; we are not prepared to discuss this at the present time. It is obvious that our findings may be the result of any combination of the preceding suggestions. But by far the most simple and logical conclusion is that there is a certain amount of the nitrogen contained in compounds capable of passing through the membrane, and that in dentin these compounds are fairly constant in percentage.

Contrary to the case of the outer fluid, the nitrogen percentage of the original dentin sample found in the inner fluid is seen to increase with the degree of completeness of demineralization, and also with the acid concentration. This indicates that, in addition to the nitrogen compounds distributed evenly throughout the system, and found in inner and outer fluids alike, there are also produced soluble nitrogen compounds which are not able to pass the membrane. This is a reasonable finding, in that it shows the formation of soluble polypeptides and not small amino acid residues during hydrolysis and demineralization.

Referring to Table II, line 12b, we see that at room temperatures, regardless of acid strength, there is a certain amount of hydrolysis accompanying demineralization. If Sample I is only about 25 per cent demineralized, it should have only about one-fourth the amount of hydrolysis, and we do see that it has a value of

inner fluid nitrogen around one-fourth the amount present in a completely demineralized sample (Sample IV). The same reasoning applies to Sample II, which is three-fourth demineralized. Concerning Sample III: we evidently had sufficient acid volume to give almost complete demineralization, but the distribution of soluble nitrogen (line 12, b and c) is an anomaly. The fact that a leak in the membrane could give this result is apparent, though had the leak been large enough to give equilibrium, there should have been nineteen times as high a percentage in the outer fluid as in the inner.

We conclude that under room temperature conditions roughly 25 per cent of the nitrogen in a sample of dentin will be in soluble compounds when the dentin is completely demineralized.

Effects of Stirring, Time, and Temperature on Demineralization.—These are simply incidental observations of effects noted during the progress of the work. Experiments were not specifically designed to prove these points, and samples were not available for them, since all the perfect teeth procurable over a period of fourteen months were required for the basic objectives. Certain things are apparent, however, and we would be remiss if we did not discuss them briefly here.

The effect of stirring is to decrease slightly the yield of protein. This is shown by results generally, but a specific example is cited. Four samples were run under identical conditions, except that only one was stirred. Protein yield for the stirred sample was 15.10 per cent, for the three unstirred samples, 15.47, 15.48, and 15.65 per cent. This indicates that about 3 per cent less protein is obtained with stirring for forty-eight hours than without stirring. As our apparatus is set up, there is sufficient surface and opportunity for the escape of salts from the vicinity of the demineralization. We have discontinued the use of stirring as being unnecessary and an added care in carrying out a demineralization.

Time of *demineralization* is a second consideration. Inasmuch as we have had molds grow in 5 per cent HCl demineralizations at the end of four to six days, we felt that three (or even better, two) days should be the maximum time to allow the dentin to stand in contact with the acid. The short time did prevent the growth of organisms; we had no cases of contamination. This meant that no preservatives and no heat sterilization were necessary—either of which could have changed the character of the protein recovered. We found that forty-eight hours (using acid of proper strength) was sufficient for the complete demineralization of dentin, yielding a product which had less than 0.60 per cent ash residue after incineration.

To show the effect of time as a variable, results of two typical demineralizations may be compared.

I.		II.	
Time	48 hr.	Time	72 hr.
Temp.	22° C.	Temp.	23° C.
Stirred		Not stirred	
Yield	15.35%	Yield	15.20%

Here is a 1 per cent difference in yield. Assuming that the yield in I is 3 per cent low due to stirring, then yield II is 4 per cent low solely because of the extra twenty-four hours in contact with the acid.

These figures, and many more like them which we have obtained, lead us to conclude that there are small effects due to stirring and time of demineralization, but that these are not significant in the range in which we have worked; mechanical errors could account for all the divergence we have seen between results with and without stirring, and at forty-eight or seventy-two hours.

Temperature, on the other hand, does have a significant effect on the results in terms of recovered protein. Considering these results:

I.		II.	
Temp.	23° C.	Temp.	29° C.
Not stirred		Stirred	
Yield	15.20%	Yield	11.97%

The yield difference is about 22 per cent; both ran seventy-two hours. In general this is similar to other results obtained, showing that temperature hastens hydrolysis of protein and lowers the yield markedly. For this reason we conclude that the use of temperatures much lower than room temperature at which we have worked in the past should possibly give increased yields of protein.

SUMMARY AND CONCLUSIONS

1. A technic is described for isolation of maximum percentages of dentinal protein by demineralization.

2. The demineralization of ground dentin is carried out with hydrochloric acid at pH 1.0 during forty-eight hours at room temperatures. Variations in acid concentration have definite effects on protein yields.

3. The protein recovered amounts to 15.5 per cent of the original weight of dentin. This protein is white, contains over 16 per cent nitrogen, and leaves less than 0.6 per cent ash on incineration.

4. Of the total amount of nitrogen in the original sample of dentin, from 97 per cent to 99 per cent has been accounted for in solutions and solid material recovered from demineralization.

REFERENCES

1. Leicester, Henry M.: Biochemistry of the Teeth, St. Louis, 1949, The C. V. Mosby Company, p. 58.
2. Bowes, J. H., and Murray, M. M.: The Chemical Constitution of Teeth, *Biochem. J.* **29**: 2,721, 1935.
3. Armstrong, W. D., and Brekhus, P. J.: Chemical Constitution of Enamel and Dentin, *J. Biol. Chem.* **120**: 677, 1937.
4. Armstrong, W. D.: Biochemical and Nutritional Studies in Relation to the Teeth, *Ann. Rev. Biochem.* **11**: 441, 1942.
5. Pincus, P.: A Sulphated Mucopolysaccharide in Human Dentin, *Nature* **166**: 187, 1950.
6. LeFevre, M. L., and Manly, R. S.: Moisture, Inorganic, and Organic Contents of Enamel and Dentin From Carious Teeth, *J. A. D. A.* **25**: 233, 1938.
7. Hess, W. C., Losee, F. L., and Neidig, B. A.: The Estimation of Some of the Amino Acids in Dentin Protein, *J. D. Res.* **29**: 664, 1950.
8. Widdowson, T. W.: Special or Dental Anatomy and Physiology and Dental Histology, Vol. I, ed. 7, London, 1946, Staples Press, Ltd., p. 128.
9. Ma, T. S., and Zuazaga, G.: Micro-Kjeldahl Determination of Nitrogen, *Indust. & Engin. Chem.* **14**: 280, 1942.

EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF THE MANDIBULAR ARCH IN AMBLYSTOMA PUNCTATUM

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INTRODUCTION

PLATT[1] gave the name *mesectoderm* to the migrating mass of ectodermal cells which are the source of origin of certain mesenchymal tissue. Since then, the origin of the visceral arch skeleton has become a special aspect of the wider problem of the origin and fate of the mesectoderm arising from the neural crest. Platt,[1] Landacker,[2] Stone,[3, 5, 6, 11] Raven,[4, 9, 10] Holtfreter,[7] and Hörstadius[15] have demonstrated that the cells of the neural crest contribute to the formation of the visceral arch skeleton. DeBeer[8] described the history of these contributions and stated that Meckel's cartilage and the teeth of Urodeles are derived from the neural crest.

Adams[12, 13] showed that the pulp of the tooth germ is derived from mesectoderm. She raised the question of whether the endoderm or the mesectoderm initiates tooth formation, or whether they are dependent, one upon the other, for some interacting force. This question is left unanswered.

Avery[14] found in the study of the *Amblystoma maculatum* that the neural crest cells migrated into the region of the future mandible and maxilla. These cells appeared intermingled with the mesodermal cells along the outer border of the stomadial collar in the future oral ectoderm. They appeared to be incorporated in the pulpal tissue of the developing tooth-forming processes which pass into the tubular dentin. He demonstrated also that alkaline phosphatase was localized in the neural crest cells under the epithelium of the future oral ectoderm as well as in the oral ectoderm itself.

The papers of Adams[12, 13] cover adequately the formation of the mouth and teeth of *Amblystoma punctatum.* She pointed out that endodermal as well as ectodermal enamel organs exist in the tooth germ of *Amblystoma punctatum,* and occasionally an enamel organ may be composed partly of ectodermal and partly of endodermal cells.

As the first of a series of studies on the mandibular arch, the following experimental procedures were performed to determine whether the axis of mandibular growth is determined at an early stage and whether the regional location of the mandibular arch has any influence on tooth formation. Healing and regeneration of the mandibular arch were also studied.

EXPERIMENTAL

Mandibular Arch Reversals.—

Group I: The mandibular arch of one side was rotated through 180 degrees on the transverse axis, thus reversing both the D-V and the A-P axes. The operations were done on embryos: twenty-five in stages 29 to 30 (Group 1a), five in stage 36 (Group 1b).

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Group II: The mandibular arch of one side of fifteen embryos (stage 29) was transplanted to the opposite mandibular arch area in fifteen embryos of the same stage, thus reversing the A-P axis only. (Fig. 1, *A*, is a photomicrograph of a transverse section through the mandibular arch of a normal embryo in stage 30.)

Fig. 1.—*A*, Transverse section through the head of *A. punctatum*, stage 30, showing the degree of differentiation of the mandibular arch at the time of operation. *B*, Photograph of larva of *A. punctatum*, showing the normal contour of the mandibular arch (three weeks after feeding stage [stage 46]).

Transplantation Experiments.—The left or right mandibular arch was removed from a donor embryo and transplanted to the region of the first to the third somite of a host embryo. Forty experiments were done on embryos in stage 29.

Healing Experiments.—Incisions were made along one boundary or along a combination of two or more boundaries (Fig. 2) of the mandibular arch in

eighty embryos (stages 29 to 30). These were made in such a way that in some only the midline was incised; in some the dorsal boundary was incised, and in others the ventral and/or posterior boundaries were cut. The arch was also completely excised and replanted.

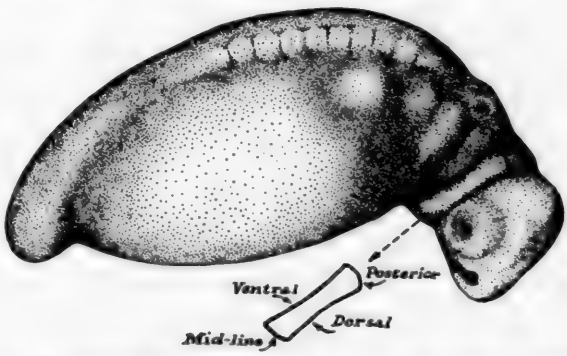

Fig. 2.—Drawing of *A. punctatum* embryo, stage 30, illustrating those borders of the mandibular arch which were incised.

Animals were sacrificed at various time intervals after stage 46 and fixed in Bouin's solutions. Serial transverse sections were prepared through the heads of all animals, and through the regions occupied by the heterotopic arch graft. All sections were stained with hematoxylin and erythrosin.

RESULTS

Group 1.—

Gross Observations: Twenty-four of the thirty animals survived through stage 46, twenty in Group 1a (operated at stage 29 to 30) and four in Group 1b (operated in stage 36).

Group 1a. Twenty-two of these animals had an abnormal mandibular arch. (Fig. 1, *B,* is a photograph of a normal larva in stage 46 +.) The arch was shorter on the operated side and failed to heal in the midline, or it was so retracted that it appeared as a more or less straight bar (Fig. 3). In nine animals the distortion was so severe that the animals could not eat, although they persistently tried to catch the Daphnia, or small white worms offered as food. Six animals were able to catch Daphnia, but could not chew or swallow them. These animals could eat small worms, however. Five animals could catch and swallow Daphnia, and apparently had normal function.

Histologic Observations: The results of microscopic studies of those animals which had the functional ability to eat in spite of the abnormal appearance of the mandible are as follows.

Except in a few cases, there was no normally located union between the maxillary arch and the mandibular arch on the operated side. In several, however, union was affected (Fig. 4, *D* and *E*). Meckel's cartilage is normally a rounded mass of cartilage around which there is a thin layer of bone to which

the teeth are attached. In some experimental animals Meckel's cartilage was distorted in such a way that it formed an L-shaped mass, extending from the area in which one would normally expect to find a Meckel's cartilage up to and including the area where one might expect to find the pterygo-quadrate cartilage. This mass also contained some teeth in a layer of bone surrounding it. The teeth were arranged in an abnormal fashion, in that their crowns pointed in a lateral direction instead of toward the oral cavity. In some animals, there extended into the oral cavity a small mass of tissue which contained numerous small teeth. There was no evidence of cartilage in this connective tissue mass (Fig. 4, *E*). On the operated side in Case 38-5 there was a mass of bone which was not associated with cartilage. This bone formation was not present on the control side of this or other control animals. The distortion in another animal (Case 38-4) capable of functioning was such that the cleft on the operated side did not persist as far posteriorly as in the other animals; however, in the mandibular arch on the operated side, Meckel's cartilage was elongated and misshapen. Here again the teeth were tilted and pointed in an abnormal direction, and they were formed not only on the medial surface, directed toward the oral cavity, but also on the lateral surface of the cartilage, which is opposite to the cavity. An irregularly shaped Meckel's cartilage intervened between the teeth and the oral cavity (Fig. 4 *D*). Other animals which had the ability to eat displayed a variation of these types of abnormality. In some, Meckel's cartilage was present and teeth were formed anterior to the maxillary-mandibular union (Fig. 4, *C*). In those animals which did not have the functional ability to eat, the cleft between the maxillary and mandibular processes extended farther posteriorly. All cases showed varying degrees of distortion of Meckel's cartilage. Frequently, the unoperated side was distorted also, especially in the more anterior areas where there was no midline fusion. In these cases the arch was shorter than normal, although Meckel's cartilage was present and tooth formation was apparently proceeding normally (Fig. 4, *A*). Occasionally the distortion on the unoperated side was almost as severe as that on the operated side, in that the cartilage was considerably elongated and seemed to fuse with the hyoid cartilages. The results of the experiments with animals in Group 1b did not vary from those just described. Apparently the type of distortion produced does not depend upon the stage at which the operation is performed.

Group II.—

Only ten of the fifteen operated animals survived, of which six displayed adequate function.

The histologic appearance of all of these animals was almost normal, although simple distortion, such as a lateral shift of Meckel's cartilage, occurred (Fig. 5).

TRANSPLANTATION EXPERIMENTS

In the majority of cases the transplant of the mandibular arch took well. The color of the transplanted arch was characteristic of the skin area of the mandible. It was lighter in color and showed few or no pigment spots as compared with the area in which it was placed (Fig. 6, *C*). In some animals small teeth could be seen growing out of the transplanted mandible (Fig. 6, *B*).

Fig. 3.—Photographs of larvae: *A*, Case 36-16; *B*, Case 36-4; *C*, Case 36-5; *D*, Case 38-13; *E*, Case 38-12, illustrating the various types of gross abnormalities produced by rotating the mandibular arch through 180 degrees (A-P and V-D axes) at stages 29 to 30 or 36. All of these animals were in stage 46 +. Compare with normal, Fig. 1, *B*.

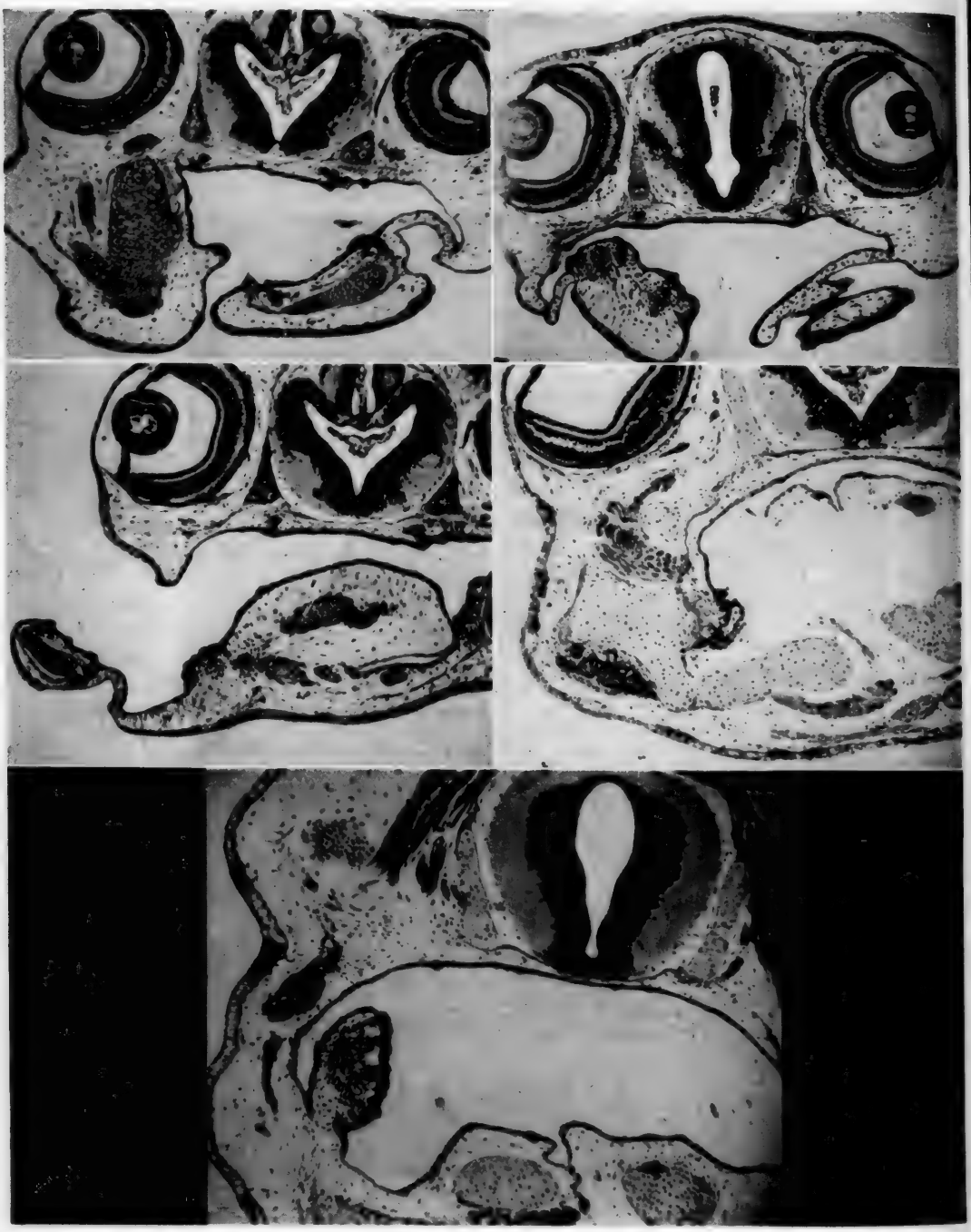

Fig. 4.—*A*, Case 38-11. Photomicrograph illustrating abnormal shape of Meckel's cartilage in right (rotated) arch. There is failure of union between the two arches. Meckel's cartilage of left arch is elongated. *B*, Case 38-10. Photomicrograph illustrating failure of union between the two sides of mandibular arch. There is no cartilage or teeth in rotated (left) arch. *C*, Photomicrograph (Case 38-3) illustrating failure of union between maxillary and mandibular arch on operated side. The arch contains cartilage and teeth. *D*, Case 38-4. Photomicrograph illustrating abnormal shape of Meckel's cartilage, which separates two groups of teeth, one in normal relation to the oral cavity, and the other on lateral aspect of cartilage. *E*, Case 38-2. Photomicrograph illustrating mass of tissue growing into oral cavity, containing teeth but no cartilage.

In others (Fig. 6, *A*), the mandibular arch transplant remained small and was apparently covered by the epithelium of the recipient area.

The mouths of the donor animals were usually so distorted that the older larva could neither catch nor swallow Daphnia, although they usually made several attempts to do so.

Histologic Observations.—

Although the microscopic findings in the graft varied considerably, there were two rather distinct features. In those animals in which no oral ectoderm and/or endoderm was removed with the transplanted mandible there was a stroma of mesenchymal tissue in which there was a single mass of cartilage. Occasionally there were two masses of cartilage separated by mesenchyme. Bone was invariably found completely or partially surrounding the cartilage. This was membrane bone and not endochondral bone. In those areas in which the oral ectoderm and/or endoderm was removed with the transplanted arch, one found not only the Meckel's cartilage surrounded by bone, but also teeth

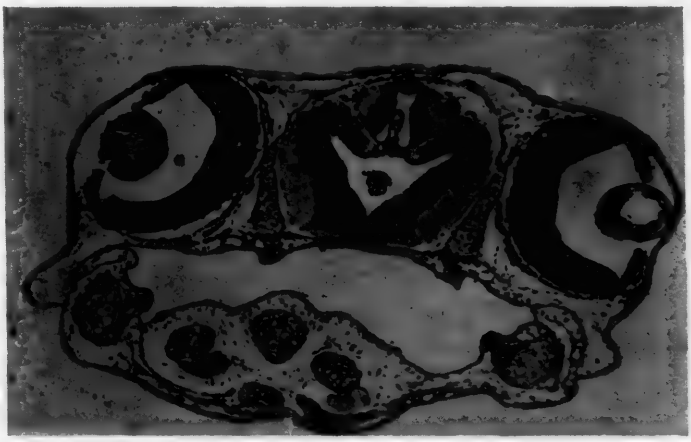

Fig. 5.—Photomicrograph of Case 45-12 R, illustrating only slight distortion of the mandibular arch in the form of a shift away from the operated (right) side.

(Fig. 7). These teeth usually arose in association with bone and were always found in the presence of oral ectoderm or endoderm. The oral ectoderm was well differentiated in some areas and appeared to contain cuboidal, columnar, or polygonal cells arranged in layers of approximately 2 to 4 cells. In some of the grafts, an oral cavity was formed, lined with epithelium through which teeth erupted (Fig. 7 *D*).

In the donor animals, complete regeneration of the removed areas of the mandibular arch did not occur. In some animals there was a partial regeneration, allowing some function. Meckel's cartilage was absent on the operated side, although simple healing did occur in this area. The typical finding was an absence of the mandibular arch on the operated side. The unoperated side showed some abnormality due to contraction of the tissues (Fig. 8).

A. *B.* *C.*

Fig. 6.—*A*, Case 37-2 R, Photograph showing heterotopic mandibular arch, covered on posterior half by skin of host area, and on anterior half by skin of donor area. *B*, Photograph of Case 37-13 R, showing heterotopic graft covered by skin of mandibular area and showing two teeth projecting from its posterior border. *C*, Case 37-18 R. Photograph illustrating grafted mandibular arch which is partially translucent, showing mass of cartilage on lateral posterior portion of graft.

A. *B.* *C.*

Fig. 7.—Photomicrographs of heterotopic arch grafts. *A*, Case 37-2 R, illustrating cartilage, bone, and tooth formation. *B*, Case 37-5 R. The heterotopic arch contains two cartilage masses. Numerous teeth surround the small "oral cavity" in center of graft. *C*, Case 37-18 R. There is no oral epithelium in this graft. A large mass of cartilage was produced, but there were no teeth. *D*, Case 37-8 R. Photomicrograph illustrating the heterotopic oral cavity contained in graft. A tooth is erupting into this cavity. Meckel's cartilage is seen above tooth. *E*, Case 37-8. Photomicrograph of another area of the heterotopic arch, showing typical tooth buds originating from the donor oral epithelium. *F*, Case 37-8. High-power photomicrograph, showing detail of heterotopic tooth. The enamel cap is clearly seen at the tip of the tooth.

Fig. 8.—Photomicrographs showing typical distortions in animals from whom one half of the mandibular arch was removed in stage 29. *A*, Case 37-1 D. Photomicrograph showing union between mandibular and maxillary arches on operated (left) side, but Meckel's cartilage is absent. *B*, Case 37-3 D. Absence of arch on operated (right) side. Duplication of Meckel's cartilage on normal side. *C* and *D*, Cases 37-13 D and 14 D. Photomicrographs showing failure of regeneration of mandibular arch on operated side, with minor distortions on unoperated side.

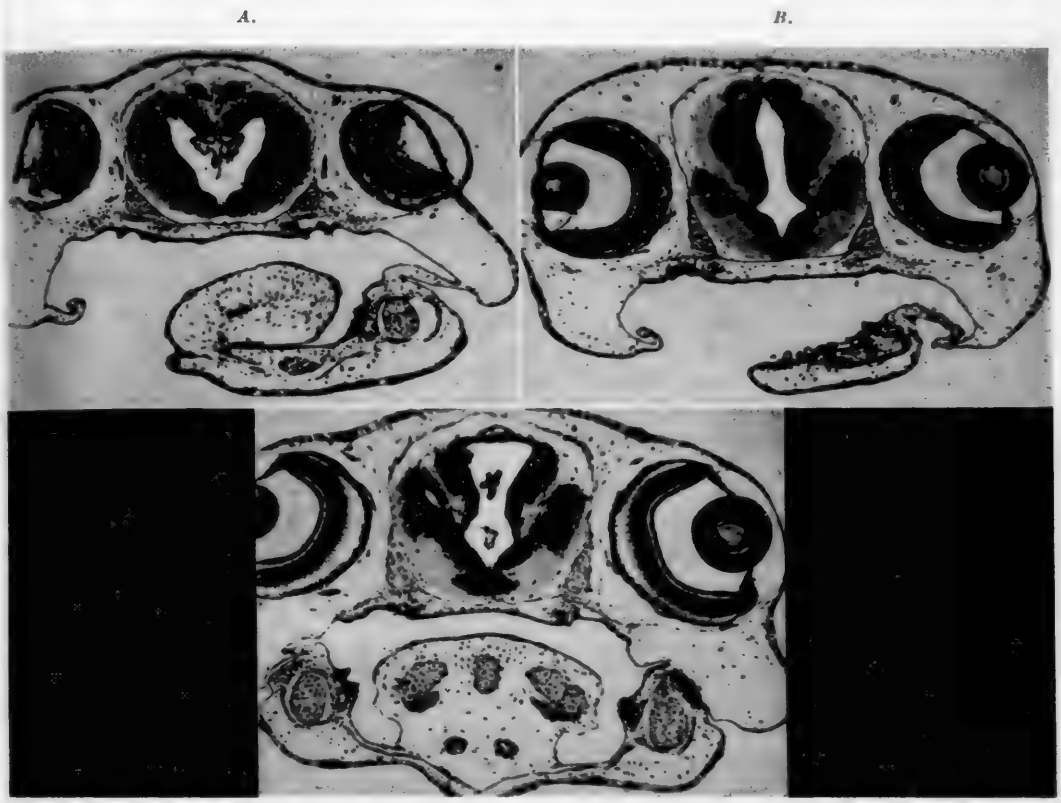

Fig. 9.—*A*, Case 46-35. Photomicrograph of a transverse section through head of an embryo following midline-posterior incisions. *B*, Case 46-34. Same treatment as *A*. *C*, Case 46-71. Normal appearance of jaws at stake 46 +. The right arch was incised in midline and posterior border at stage 31.

HEALING EXPERIMENTS (FIG. 2)

Of the eighty animals used in this experiment, sixty survived through stage 46, and only ten showed gross abnormalities. Seven of these occurred in the group with midline-posterior cuts, and one in each of the following groups: those with midline-dorsal cuts, arch incised, and arch excised and replaced. In one case in which the arch was excised and replaced, the arch was inadvertently pushed into the pharyngeal cavity during bridging.

Microscopic examination of the abnormal animals previously referred to showed changes similar to those found in those animals in which the mandibular arch was removed on one side. There was almost a complete failure of growth or healing of the mandibular arch, especially when midline and posterior incisions were made (Fig. 9).

SUMMARY AND CONCLUSIONS

The unilateral rotation of the mandibular arch through 180 degrees (reversal of both A-P and D-V) resulted in a distortion of the arch. Whereas all of the

elements normally found in the region were still present, their topographic arrangement was disturbed. Healing in this region was also abnormal. Reversing the A-P axis only did not result in such severe abnormalities.

When the mandibular arch was transplanted to the anterior somite area, its development varied considerably. If the oral epithelium was included in the graft, teeth also developed. The donor embryo, however, did not regenerate a new mandibular arch. These findings lend support to the observations of Stone and others that the cartilages and teeth of the mandibular arch are derived from the cells of the neural crest.

When incisions were made in the midline and posterior margins of the mandibular arch, gross abnormalities developed in a large percentage of the embryos so treated. However, healing occurred normally in almost all cases when incisions were along the other margins of the mandibular arch.

The results of all of these experiments show that the mandibular arch does not possess the power of complete restitution.

REFERENCES

1. Platt, Julia B.: Ontogenetische differenzirung des Ektoderms in Necturus, *Arch. f. Mikr. Anat.* **43**: 991, 1894.
2. Landacker, F. L.: The Fate of the Neural Crest in the Head of Urodeles, *J. Comp. Neurol.* **33**: 1, 1921.
3. Stone, L. S.: Experiments Showing the Role of Migrating Neural Crest (Mesectoderm) in the Formation of Head Skeleton and Loose Connective Tissue in Rana pulustris, *Arch. f. Entwckingsmechn. d. Organ.* **118**: 40, 1929.
4. Raven, C. P.: Zur entwicklung der ganglienleiste, I. Die kinematik der ganglienleistenentwicklung beiden urodelen, *Arch. f. Entwckingsmechn. d. Organ.* **125**: 210, 1931.
5. Stone, L. S.: Experiments on the Development of the Cranial Ganglia and the Lateral Line Sense Organs in Amblystoma punctatum, *J. Exper. Zool.* **35**: 421, 1922.
6. Stone, L. S.: Further Experiments on the Extirpation and Transplantation of Mesectoderm in Amblystoma punctatum, *J. Exper. Zool.* **44**: 95, 1926.
7. Holtfreter, J.: Die totale Exogastrulation, eine Selbstablösung des Ektoderms vom Entomesoderm, *Arch. f. Entwickingsmechn. d. Organ.* **129**: 669, 1933.
8. deBeer, G. R.: *The development of the vertebral skull*, Oxford, 1937, Clarendon Press.
9. Raven, C. P.: Zur entwicklung der Ganglienleiste. II. Ueber das differenzierungsvermögen des Kopfganglienleistenmaterials von Urodelen, *Arch. f. Entwckingsmechn. d. Organ.* **129**: 179, 1933.
10. Raven, C. P.: Zur entwicklung der Ganglienleiste. III Die induktionsfähigkeit des Kopfganglienleistenmaterials von Rana fusca, *Arch. f. Entwckingsmechn. d. Organ.* **130**: 517, 1933.
11. Stone, L. S.: Transplantation of Hyobranchial Mesentoderm, Including the Right Lateral Anlage of the Second Basibranchium, in Amblystoma punctatum, *J. Exper. Zool.* **62**: 109, 1932.
12. Adams, Amy E.: An Experimental Study of the Development of the Mouth in the Amphibian Embryo, *J. Exper. Zool.* **40**: 311, 1924.
13. Adams, Amy E.: Some Effects of Removal of Endoderm From the Mouth Region of Early Amblystoma punctatum Embryos, *J. Exper. Zool.* **58**: 147, 1931.
14. Avery, J. K.: Possible Ectodermal Origin of the Odontoblast, *J. D. Res.* **29**: 663, 1950.
15. Hörstodius, S.: The Neural Crest: Its Properties and Derivatives in the Light of Experimental Research, London, 1950, Oxford University Press.

THE ORIGIN AND DISTRIBUTION OF THE LABIAL AND BUCCAL GLANDS IN THE HUMAN FETUS

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THE development of the accessory salivary glands has received scant attention in the embryologic literature. It is generally agreed that they arise later than the major salivary glands. Of the latter, the epithelial primordia appear first for the submandibular and parotid glands in embryos of the sixth week of development (13 to 15 mm. embryos). The major and minor sublingual glands arise a little later, in embryos varying between 19 and 25 mm. crown-rump length, i.e. during the eighth week.

The available information on the time of appearance of the small oral glands varies. Arey[1] stated that they develop from multiple epithelial buds "at about three months." According to Fischel[2] and Broman[3] they arise during the fourth month. Detailed studies on the specific locations of the various aggregates of such epithelial primordia apparently have not been made.

The accessory salivary glands usually are subdivided into groups and described as labial, buccal and molar, retromolar, palatine, and lingual glands.

The locations and relationships of the labial and buccal glands to the orbicularis oris and buccinator muscles in the adult are well known (Toldt[4] and Sicher[5]). Some of these glandular groups protrude outward from the submucosa through the interlacing fibers of those muscles. Other glandular aggregates form a fairly compact body, the retromolar gland within the retromolar pad.

The labial glands can be felt as nodular masses in the lips. There are very few, if any, buccal glands lateral to the corner of the mouth or in the occlusal plane. The earlier portrayal of their arrangement by Toldt (a figure reproduced in Morris' *Human Anatomy*) appears more accurate than that of Sicher.

A special functional significance has been ascribed to these glands. Kirk[6] stated, as early as 1902, that the labial and buccal glands may secrete a saliva which causes enamel erosion. He believed "that dental erosion was a constitutional disorder with a local manifestation and that the local expression was the solvent action upon tooth structure of an altered mucus from certain of the buccal mucous glands." Kirk concluded from his study that the solvent produced by abnormal buccal glands was either acid sodium phosphate or acid calcium phosphate.

We consider it quite possible that such a causative relationship between enamel erosion of the incisors and abnormal secretion by the labial glands may exist and that the early views of Kirk deserve further study. Estimates have been made that enamel erosion affects about 5 per cent of the population.

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A suggestion of the physiologic significance of the labial glands is also contained in the textbook of dental histology by Schour and Noyes.[7] They describe the glands as either mucous, serous, or mixed and state that "the secretion of these glands is probably much more important than has been supposed."

MATERIAL

Six human fetuses varying in age from the third to the fifth month were studied. The facial portions of the heads were embedded in paraffin or in celloidin and serially sectioned. The full number of sections was mounted and stained for the younger stages. Every tenth section of the older stages (fifth month of fetal development) was mounted. As these larger preparations were embedded in celloidin and the complete set of sections kept in alcohol between filter papers, we had the opportunity of studying additional sections whenever the need arose. In some head preparations the lips and cheeks were resected from the skeletal portions and embedded as soft tissues. The full facial portion of the head was sectioned of the youngest stage (3.2 months). Partial preparations were made of the older heads by freezing and trimming them to appropriate size with a fine-toothed bandsaw. Decalcification in nitric acid was checked by repeated x-ray films. The partial preparations included one side of cheeks and lips and extended by 1 cm. beyond the midsagittal plane. Over one-half of the tongue, hard and soft palate, nasopharynx, and base of the skull was thus retained. The paraffin sections were cut at 10 microns, the celloidin sections at 20 microns. The ages of the fetuses were calculated in lunar months from the measured crown-rump length (CRL).

OBSERVATIONS

The origin of the labial and buccal glands depends on the establishment of the vestibular epithelial plate. Originally there is a single primordium for the lips, cheeks, and jaws. During the sixth week of development the dental lamina arises from the oral epithelium. It is the forerunner and formative element of the tooth buds. A little later, a solid epithelial plate grows inward from the base of the dental lamina and leads to a gradual separation of the lips and cheeks from the jaws proper. This epithelial invagination in the form of an arched plate is the labiogingival or vestibular lamina. During the third month of development the central cells of this epithelial plate degenerate and the lamina itself gradually becomes transformed into a moat-like sulcus. This constitutes the oral vestibule (sulcus labialis or vestibularis). It is also called sulcus labiogingivalis or sulcus alveolo-labialis. At the anteromedial portions of the upper and lower jaws the vestibular sulcus remains low. The mesenchymal tissue between the vestibular grooves of either side forms here the upper and lower labial frenulum.

The musculature of the lips and cheeks, e.g., the orbicularis oris and buccinator muscles, differentiate even earlier, during the second embryonic month. Their arrangement and the course of their fibers in regard to the labial and buccal submucosa are easily recognized between the third and fifth fetal months.

The labial glands arise early in the third month of human development. The primordia of the superior labial glands develop from the upper vestibular lamina which is still a solid epithelial plate. Therefore, there is no oral vestibule early in the third month. Canalization of the vestibular plate is just beginning posteriorly and gradually extends forward. Further developmental characteristics of this stage are the ossification centers of the premaxilla, maxilla, and palatine bones. The highest epithelial buds of the superior labial glands arise from the uppermost portion of the vestibular lamina. They lie directly in front of the primordia of the first and second incisors, at the level of a horizontal plane which passes through the upper dental lamina which carries four toothbuds. The palate and the opening of the Eustachian tube lie in the same plane. There are four to six such glandular buds in any one section. At slightly lower levels, additional primordia of superior labial glands arise more laterally from the vestibular lamina, opposite the third toothbud but in front of the anterior margin of the buccinator muscle.

The zone of differentiation of upper labial glands, at this early stage of development, extends through thirty sections. It measures about 300 microns (0.3 mm.) in vertical height. The number of individual glandular buds is difficult to establish without three-dimensional reconstructions, as the same glandular outgrowth may be encountered in more than one section. Many of these primordia terminate in a slight nodular enlargement from which the glandular acini arise during the subsequent weeks. All arise from the deepest layer (stratum germinativum) on the labial side of the vestibular lamina. At this stage, all the buds of the future superior labial glands lie well above the lower border of the upper gingiva.

The inferior labial glands also arise early in the third fetal month. Their epithelial buds differentiate both from the labial epithelium above the upper border of the lower jaw and from the inferior vestibular lamina in front and below that border. There is no open inferior vestibule at this stage of development. The highest epithelial buds of the future inferior labial glands lie in front of the tip of the tongue, well above the inferior dental lamina. The lowest glandular outgrowths occur from the temporary bottom of the vestibular lamina. The zone of differentiation of these earliest glandular sprouts extends through fifty sections (500 microns or 0.5 mm.). Thirty of these sections concern horizontal planes above the upper border of the lower jaw. A majority of the early epithelial primordia of inferior labial glands, therefore, arise directly from the oral (labial) epithelium. This might be due to an earlier separation of the lower lip from the common primordium with the lower jaw.

Fig. 1 is a low-power reproduction of a horizontal section through the tongue, lower jaw, and lip of a 3.2-month human fetus (CRL = 6.5 cm.). It shows the lateral group of epithelial buds of inferior labial glands. The dental lamina was encountered anteriorly and dorsolaterally because the upper border of the mandibular primordium is concave. The intermediate portion is occupied by a thick oral epithelium which extends forward as the inferior vestibular lamina. The glandular buds lie near the anterior border of the buccinator

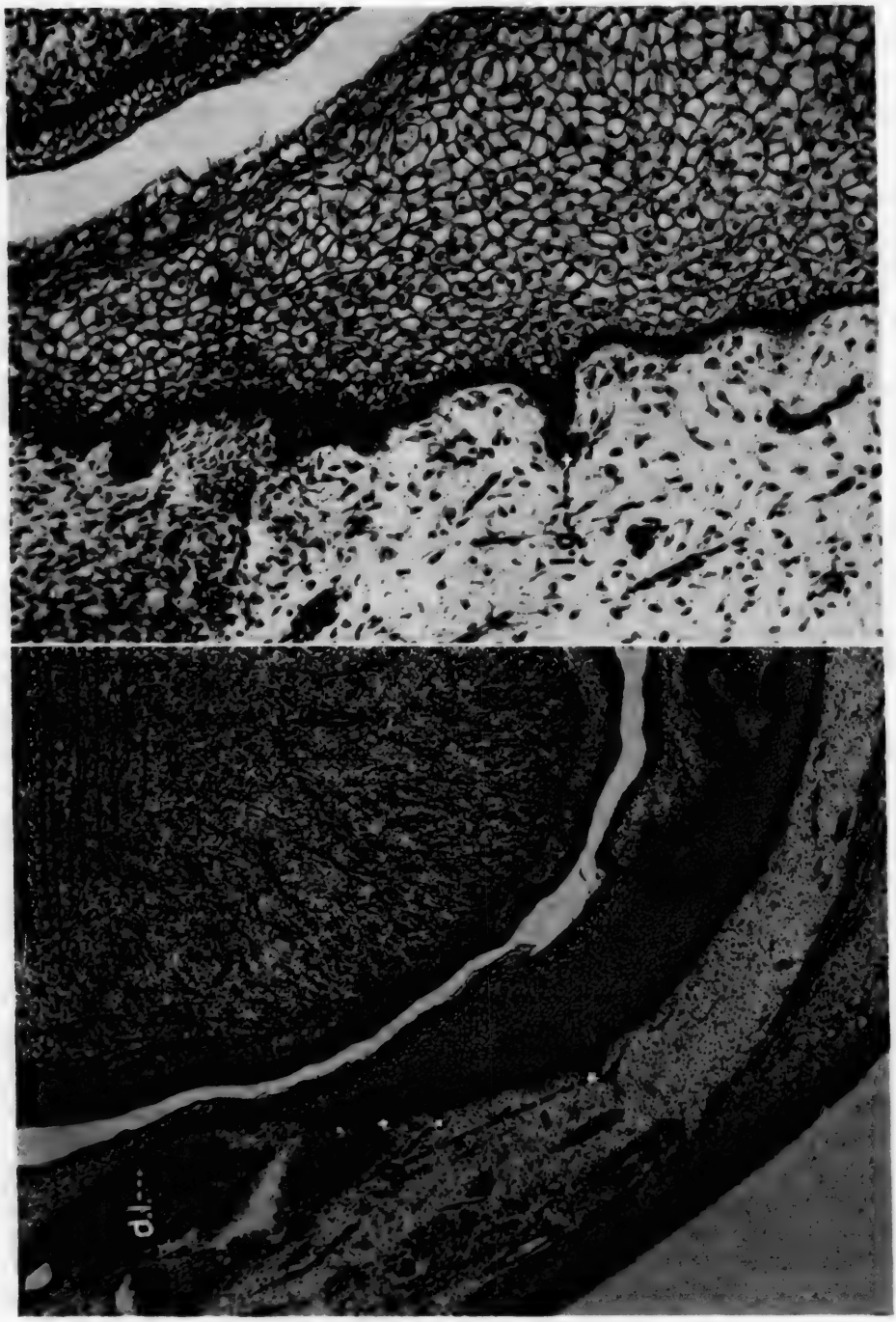

Fig. 1.

Fig. 2.

Fig. 1.—Horizontal section through the tongue, mandible, and lip of a 3.2-month human fetus. *l.g.*, Solid epithelial primordia of inferior labial glands; *d.l.*, dental lamina. (Magnification approximately $\times 43$.)

Fig. 2.—Epithelial buds of inferior labial glands. Human fetus, 3.2 months. High-power detail of Fig. 1. *l.g.*, Labial gland primordium. (Magnification approximately $\times 185$.)

muscle. Fibers of the orbicularis oris muscle are easily identified within the lower lip. Fig. 2 represents a higher magnification of the same epithelial primordia of a group of inferior labial glands in a 3.2-month fetus.

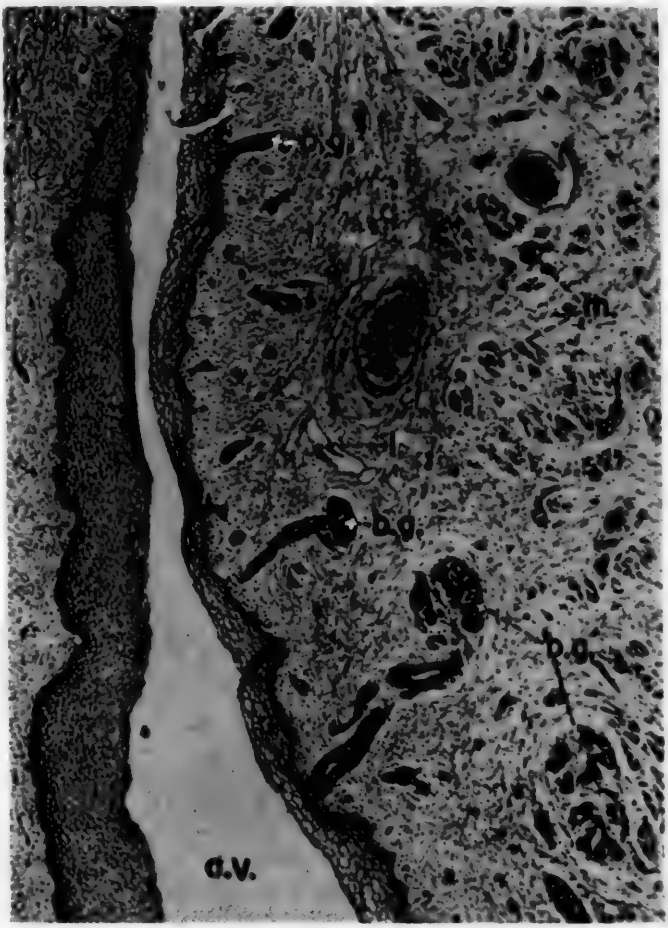

Fig. 3.—Developing buccal glands. Human fetus, 4.6 months. *b.g.*, Tubular branching primordia of glands in the submucosa; *l.*, lymph vessel with valve; *m.*, differentiating orbicularis oris muscle; *o.v.*, oral vestibule. Sagittal section. (Magnification approximately ×62.)

During the fourth fetal month the canalization of the original glandular outgrowths and their ramification occur. Glandular ducts with a lumen and the earliest glandular acini thus become established. The acini remain closely grouped together and the individual labial glands rapidly acquire their characteristic form and locations (Fig. 3). The vestibular lamina meanwhile has "opened up" by degeneration of its central cells so that a definite oral vestibule now exists. The glands shown belong to the upper buccal group as the particular section passes lateral to the corner of the mouth. The branching of the

tubular primordia of these glands and their compound nature are clearly visible. All the glandular nodules lie in the submucosa, inside of the differentiating facial muscles.

The distribution of glandular acini of the superior and inferior labial glands is shown in Fig. 4 This is a graphic reconstruction from tracings of serial sections of a human fetus of 5.2 months (CRL = 16.2 cm.). The facial portion of the head was embedded in celloidin and every tenth section stained and mounted. The superior and inferior alveolar (gingival) borders, the extent of the oral vestibule, some tooth germs, the mouth, and the parotid duct were projected in their correct locations and respective relationships. The locations and distribution of the superior and inferior labial glands are marked by short heavy lines.

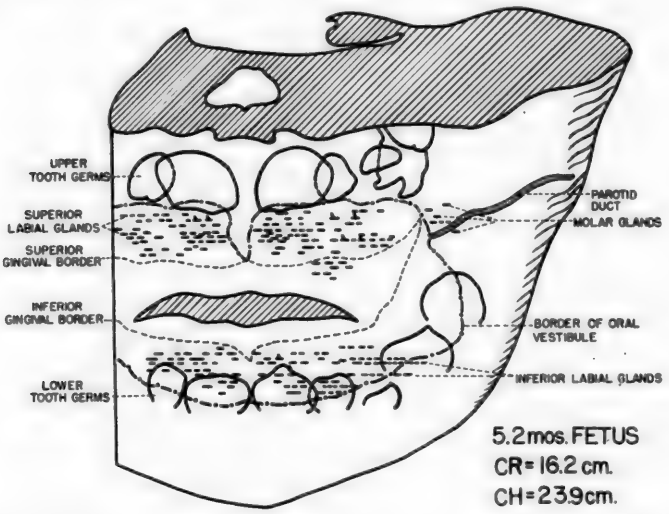

Fig. 4.—Graphic reconstruction (projection) from serial sections of the labial and the left molar glands. Human fetus, 5.2 months.

As the gaps between the studied sections amounted to 0.2 mm., the plotting of the glandular acini is incomplete and their full continuity is not shown. An over-all grouping, nevertheless, is recognizable and the general distribution pattern of these small glands is correctly given. It is seen that at this stage of development all the superior labial glands are located in the upper lip at a lower level than the deciduous tooth germs. Most of them empty into the upper oral vestibule above the lower margin of the gingiva. A single group of glands extends below that level in the left upper lip. The superior labial frenulum is free of glands. The upper oral vestibule probably expands further craniad during later development and additional glands may arise from the ascending fornix. The average vertical extent of the upper and lower vestibules (fornix to gingival border) is nearly the same, i.e., 2.2 millimeters. The distribution of the inferior labial glands shows that the lower groups lie partly in front of the lower tooth germs and partly above them.

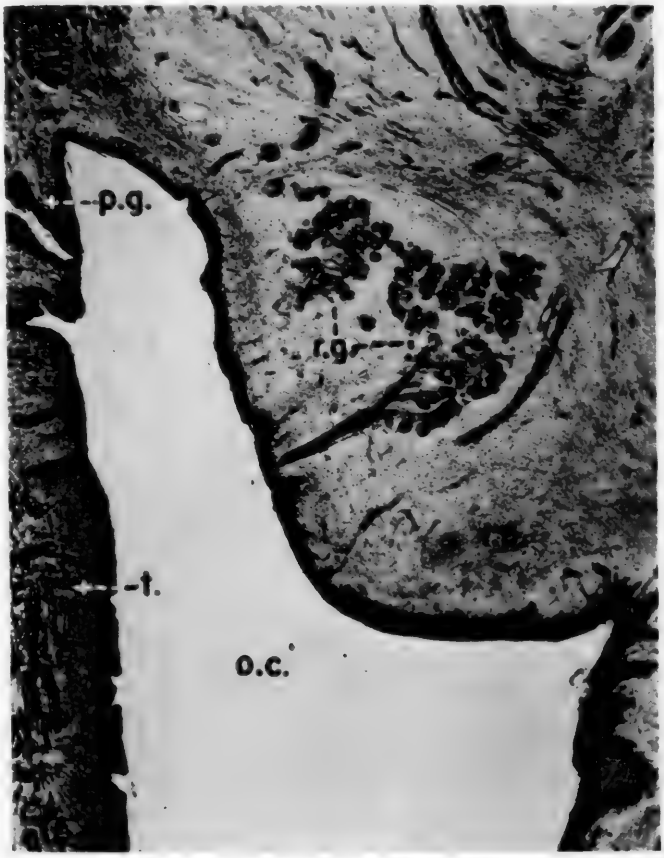

Fig. 5.—Retromolar glands (*r.g.*) and papilla (*r.p.*) of a 5.2-month human fetus. *t.*, Tongue; *o.c.*, oral cavity; *p.g.*, palatoglossal fold. (Magnification approximately ×32.)

The primordia of a group of "molar" glands are closely associated with the termination of the parotid duct in the oral vestibule. The nomenclature of these glands is not uniform in various descriptive texts. Perhaps they should be recognized rather as posterior buccal glands. In any case they are not to be confused with the retromolar glands which are located at the retromolar papilla near the base of the mandibular ramus.

Fig. 5 illustrates the location and degree of development of the true retromolar glands in a 5.2-month fetus. The photographic field represents a section through the left retromolar papilla. It is a relatively broad fold at the posterior end of the inferior gingival primordium, lying directly in front of the medial plate of the mandibular ramus. At this stage of development the ramus consists of two anteriorly diverging bony plates. The posterior end of the inferior dental lamina is continuous with the epithelial covering of the retromolar papilla and the last molar tooth germ develops from it between the diverging bony plates of the mandible. The relatively well-developed retromolar glands oc-

cupy the medial aspect of the papilla. They are compound tubular glands and extend upward and medially toward the soft palate where the somewhat simpler glandular primordia become the palatine glands. On the inside of the medial bony plate lies the lingual nerve, directly posterior to the retromolar papilla. Lateral to the papilla occurs the attachment of the buccinator muscle on the lateral plate of the developing mandible. The retromolar glands develop relatively late, during the latter part of the fourth or early in the fifth month.

The area of the retromolar papilla, at this stage of development, is also directly lateral to the palatoglossal fold which contains the corresponding muscle. The palatine tonsil with its epithelial crypts and accumulations of lymphoid tissue begins its definite differentiation at this stage, posteromedial to the palatoglossal fold.

The locations and degree of development of the superior and inferior labial glands in the same fetus (5.2 months) are illustrated respectively by Figs. 6 and 7. The racemose nature of the glandular primordia of the superior labial glands is evident in the horizontal section which passes through the left upper lip and gingiva near the superior frenulum. The gingival tissue portrayed in Fig. 6 is near the lower border of the developing upper gingivae. The inferior labial glands shown in Fig. 7 appear to be larger or further differentiated than their upper counterpart. The horizontal section shown passes through the inferior vestibule above the level of the developing inferior frenulum. The tooth germs of the inferior medial incisors are visible and between them, in the midsagittal plane, an epithelial remnant of the line of fusion between the lower gingivae is visible.

In order to compare the foregoing description of the development of minor salivary glands with that of a major one, Fig. 8 portrays the degree of differentiation of the left submandibular gland in the same fetus of 5.2 months. Cross sections of the anterior facial vein and of a developing submandibular lymph node are seen to lie laterally adjacent to it. The gland consists already of many lobules, and the encapsulation by connective tissue as well as septal formations is well under way. The glandular branches are still solid buds which retain their faculty of further division and the ultimate production of terminal acini.

DISCUSSION

Our observations on the origin of some of the minor salivary glands have shown that their earliest appearance is related to the time of separation of the lips from the jaws. This concerns the development of a labiogingival epithelial plate and its subsequent opening up in forming the oral vestibule. These processes occur early in the third fetal month. At that time the fusion of the palatal components has already occurred and the development of the major salivary glands is well under way.

The truly embryonic period of development in man is terminated at the end of the second month and is followed by the fetal period. All the primordia of major organs become established during the first phase. It is also the time when the essential developmental events occur that lead to normal or abnormal

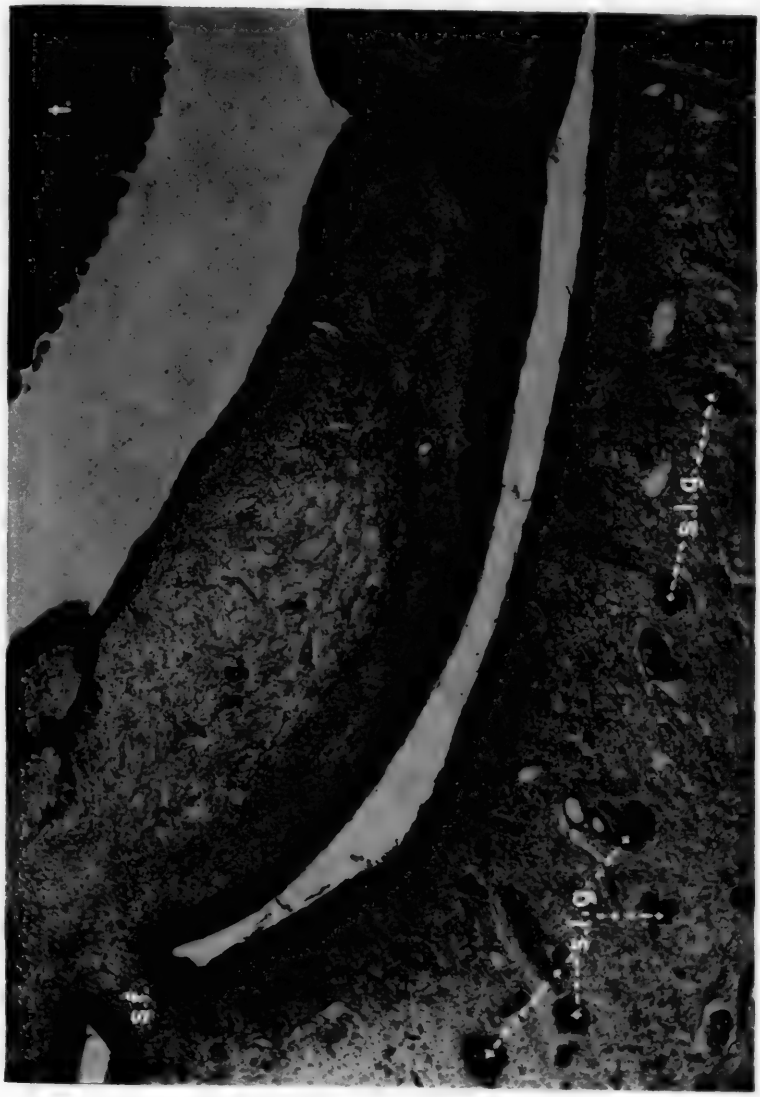

Fig. 6.—Superior labial glands (*s.l.g.*) of a 5.2-month human fetus; *s.f.*, superior frenulum; *s.g.*, superior gingiva; *t.*, tongue. (Magnification approximately ×35.)

Fig. 7.—Inferior labial glands (*i.l.g.*) of a 5.2-month human fetus; *o.v.*, oral vestibule; *i.t.g.*, inferior medial incisor tooth germs; *m.*, orbicularis oris muscle. (Magnification approximately ×38.)

relationships between organ and organ systems. The major salivary glands are strictly of embryonic origin, whereas the minor glands arise early in the fetal period. This is a period of finer histodifferentiation, of the establishment of minor organs, and primarily of growth of the earlier primordia. The development of the minor salivary glands characterizes, therefore, that period of detail differentiation. The whole range of the numerous accessory salivary glands,

Fig. 8.—Submandibular gland and adjacent structures of a 5.2-month human fetus; *a.f.v.*, anterior facial vein; *l.n.*, developing submandibular lymph node; *c*, differentiating capsule and septa of the gland. (Magnification approximately ×43.)

i.e., the labial, buccal, molar, retromolar, as well as the palatine and lingual glands, arises early in fetal life. The retromolar glands appear last, early in the fifth month. During the third and fourth month occur also the differentiation of the glands in the nasal mucosa and of the primordium of the maxillary sinus.

The discrepancies in the literature concerning the time of origin of the accessory salivary glands are minor. Our observations agree with Arey.[1] Differences of opinion as to whether they arise during the third or fourth month

probably are related to variable age determinations of the specimens. There may also be some individual variation. We paid special attention to the pattern of distribution of the early primordia of the upper and lower labial glands. The later functional significance of these glands to enamel erosion of the incisors is a distinct possibility. While studies on fetal material obviously cannot establish factors in support of such views, they at least elucidate the topography of these glands during their earliest developmental history.

The further histologic differentiation of the major as well as of the minor salivary glands proceeds throughout the later fetal months. The process probably is not completed until some time after birth. As long as the terminal branches of the glandular primordia remain solid, the potentiality of producing additional buds is retained. This faculty is lost after canalization of the ducts has occurred and the terminal acini have acquired lumina. Mucous and serous cells thereupon become recognizable in the glandular acini.

SUMMARY

The superior and inferior labial glands arise early in the third month of human development. They develop from epithelial buds of the vestibular epithelial plate before there is an open alveolo-labial sulcus. A group of buccal or molar glands arises at the same time. They are associated with the termination of the parotid duct and lie, therefore, in the mucosa of the upper cheek. The retromolar glands develop later, early in the fifth fetal month. They are located on the medial side of the retromolar papilla. In the fetus with its primitive mandible and low ramus the papilla is a relatively broad fold, directly continuous with the soft palate.

The major salivary glands (submandibular, parotid, and sublingual) arise earlier, usually in that order, during the latter part of the second embryonic month.

The topographic relations of the labial and molar glands are portrayed in a fetus of the fifth month.

The authors wish to express their appreciation to Mr. L. A. Toriello of the Illustration Studios of the University of Illinois, College of Medicine, for his skillful assistance in preparing the photomicrographs.

REFERENCES

1. Arey, L. B.: Developmental Anatomy, ed. 4, Philadelphia, 1940, W. B. Saunders Company.
2. Fischel, A.: Entwicklung des Menschen, Berlin, 1929, Julius Springer.
3. Broman, I.: Die Entwicklung des Menschen vor der Geburt, München, 1927, J. F. Bergmann.
4. Toldt, C.: Anatomischer Atlas, Wien, 1914, Urban und Schwarzenberg.
5. Sicher, H.: Oral Anatomy, St. Louis, 1949, The C. V. Mosby Company.
6. Kirk, E. C.: The Clinical and Chemical Study of a Case of Dental Erosion, *D. Items Interest* **24**: 511, 1902.
7. Schour, I., and Noyes, H. J.: Dental Histology and Embryology, ed. 5, Philadelphia, 1943, Lea and Febiger.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the New York Section

School of Oral and Dental Surgery, Columbia University, New York
June 4, 1951

Frances Krasnow, *Editor*

1. The Lipids of Dental Tissues: A Preliminary Study. *Basil C. Soyenkoff, Bernard K. Friedman, and Margaret Newton, New York University College of Dentistry, New York, N. Y.* The lipid content of dentin is of interest in connection with the transmission of stimuli and the penetration of anesthetics; however, there is a lack of information on the presence and chemical nature of lipids in the dental tissues. An initial study was therefore made with 300 Gm. of mixed dental tissue which contained 82 per cent dentin and the remainder enamel, with less than 0.05 per cent of cementum. About 300 mg. of chloroform-soluble material has so far been obtained by successive extraction with several solvents, and the presence of sterols and (on saponification) fatty acids has been established. Among the other constituents appear to be aminophospholipids and related substances, whose nature is being investigated. Confirmatory studies are being made on relatively small amounts of root dentin, and progress to date will be reported.

2. The Role of the Cation in the Sodium Chloride Activation of Salivary Amylase. *Leon H. Schneyer, Dental Department of Montefiore Hospital, New York, N. Y.* Salivary amylase, dialyzed free of salts, shows virtually no amylolytic action. The presence of 10^{-4} N sodium chloride restores activity. The activating component is considered to be the chloride ion. The results of this investigation indicate that, at temperatures near the optimum for amylase activity, the cation increases the degree of activation by chloride and does this by slowing the denaturation of the enzyme. A comparison of amylase activity in the presence of 3.6×10^{-2} and 3.6×10^{-3} N sodium chloride shows that with the greater NaCl concentration (1) amylase activity is greater at all temperatures within the 35 to 55° C. range; (2) the temperature optimum is at a higher value; and, (3) the rate of increase in activity as the temperature is raised to the optimum is greater, and the rate of decrease in activity is slower as the temperature is raised beyond the optimum. These effects indicate that NaCl slows the reversible denaturation of salivary amylase. Sodium chloride also reduces the irreversible denaturation of salivary amylase produced by temperatures above the optimum. The effects of increased sodium chloride concentrations can be obtained with lower concentrations of sodium chloride in the presence of sodium phosphate. Sodium phosphate alone is virtually without activating action. The effects of sodium chloride on the temperature response of amylase are, therefore, due to the sodium ion.

3. Acid Retention in Gold Castings: A Preliminary Report. *Chester J. Henschel, Eastern Graduate Research Foundation, New York, N. Y.* Experiment: 1—to determine the accuracy of original hypothesis explaining occasional otherwise seemingly inexplicable cast inlay or crown failure, i.e.: that pickling acid, trapped in one or more narrow mouthed vacuoles, slowly leaches out, dissolves cement and allows ingress of fermentable carbohydrates. 2—to ascertain

how neutral castings may be assured. One hundred average cast inlays were a.—quenched in HCl, b.—soaked in HCl. In either or both instances, retained chlorides were found even after eight minutes of agitation in running (distilled) water. Post-acid treatment by casual washing and soaking in sodium bicarbonate solution gave similar retained chlorides. It required more than 4 hours of bicarbonate immersion to produce neutral castings. Castings under bicarbonate for 8 hours retained bicarbonate even after thorough washing. The same castings quenched in water after acid bath retained no chlorides. The distilled water in which the castings were soaked after washing or bicarbonate showed a drop of 2.5 to 2.8 pH and nearly 0.3 mg. chlorides per ml. It is proposed that castings be heated to a cherry red heat and quenched in 70 per cent alcohol to brighten them and dissipate retained acid. The alternative is bicarbonate bath for 4 to 8 hours.

4. THE ROLE OF VITAMIN B_{12} IN THE TREATMENT OF GLOSSOPYROSIS: A PRELIMINARY REPORT. *Samuel C. Miller, Norman E. Alderman, and Sheldon Ross, New York University, College of Dentistry, New York, N. Y.* Published as Abstract 146 of the Proceedings of the I. A. D. R. (p. 515, this issue).

5. PRACTICAL PRESCRIPTION PROCEDURES IN VITAMIN THERAPY: A PRELIMINARY REPORT. *Samuel Charles Miller, Harry Roth, and Marvin Simring, New York University, College of Dentistry, New York, N. Y.* Published as Abstract 147 of the Proceedings of the I.A.D.R. (p. 516, this issue).

Erratum

In the paper by S. Wah Leung, "A Demonstration of the Importance of Bicarbonate as a Salivary Buffer," (*J. D. Res.* **30**: 403, June, 1951) the parenthetical phrase on the twelfth line from the bottom of page 407 should read "$H_2CO_3^-$" and the formula beginning the eighth line on page 408 should read "$H_2CO_3^-$" (not $HCO_3^-H_2CO_3$).